STUDIES ON POTASSIUM SOLUBILIZING BACTERIA

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

In

AGRICULTURAL MICROBIOLOGY

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DECEMBER, 2007

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1. INTRODUCTION

Potassium is one of the essential macronutrient and the most abundantly absorbed cation in higher plants. The introduction of high yielding varieties and hybrids during green revolution and with the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate. As a consequence, potassium deficiency is becoming one of the major constraints in crop production, especially in coarse textured soils. Even in fine textured soils the available fraction is low compared to total K in them, crops do respond to K fertilization in soils with high available K.

Potassium plays an important role in the growth and development of plants. It activates enzymes, maintains cell turgor, enhances photosynthesis, reduces respiration, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis. In addition to plant metabolism, potassium improves crop quality because it helps in grain filling and kernel weight, strengthens straw, increases disease resistance and helps the plant better to withstand stress.

Potassium is the fourth most abundant nutrient constituting about 2.5 per cent of the lithosphere. However, actual soil concentrations of this nutrient vary widely ranging from 0.04-3.00 per cent (Sparks and Huang, 1985).

Plant can take up potassium only from the soil solution. Its availability is dependent upon the K dynamics as well as on total K content.

There are three forms of potassium found in the soil *viz.,* soil minerals, non-exchangeable and available form. Soil minerals make up more than 90 to 98 per cent of soil potassium. It is tightly bound and most of it is unavailable for plant uptake. The second is non-exchangeable potassium which acts as a reserve to replenish potassium taken up or lost from the soil solution. It makes up approximately 1 to 10 per cent of soil potassium.

The third type is available potassium which constitutes 1 to 2 per cent. It is found either in the solution or as part of the exchangeable cation on clay mineral.

Among three different forms of potassium in soils, the concentrations of soluble K in soils are usually very low but the highest proportion of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994). A significant share of soil potassium occurs in unavailable form in soil minerals such as orthoclase and microcline (K-feldspars).

Although K deficiency is not as wide spread as that of nitrogen and phosphorus, many soils which were initially rich in K become deficit in due course due to heavy utilization by crops and inadequate K application, runoff, leaching and soil erosion (Sheng and Huang, 2002). Potassium deficiency symptoms usually occur first on the lower leaves of the plant and progress towards the top as the severity of the deficiency increases. One of the most common signs of potassium deficiency is the yellow searching or firing (chlorosis) along the leaf margin. In severe cases of potassium deficiency the fired margin of the leaf may fall out. Potassium deficient crops grow slowly, poorly developed root systems and stalks are weak, and lodging of cereal crops.

Microorganisms play a key role in the natural K cycle. Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. There are considerable population of K solubilizing bacteria in soil and rhizosphere (Sperberg, 1958). Silicate bacteria were found to dissolve potassium, silicon and aluminium from insoluble minerals (Aleksandrov *et al*., 1967). It has been reported that most of potassium in soil exists in the form of silicate minerals. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch *et al*., 1985).

Mineral potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan *et al*., 1996). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, illite and orthoclases through production and excretion of organic acids (Fridrich *et al*., 1991).

The special focus on K solubilizer was due to the fact that potassium is one of the major nutrient required by all crops. It is a key element in many physiological and biochemical processes.

The available potassium status in Indian soils has been categorized into three classes where 21 per cent districts fall in low, 51 per cent in medium and 28 per cent in high potassium content soils (Ghosh and Hasan, 1980). The available potassium in major soils of Karnataka is low to medium. Red soils (10.6 m.ha), black soils (6.2 m.ha) and lateritic soil (2.0 m.ha) are the major soil groups in the state. In general, black soils are high, red soils medium, and lateritic soils low in available K. Lateritic, shallow red and black soils have been found to show decline in K fertility over the years under intensive cultivation and imbalanced fertilizer application.

Since K is a costly nutrient, India ranks 4th in consumption of potassium fertilizers. On an average 1.7 million tones of K is being imported annually (Anonymous, 2003). Successful identification of an elite microbial strain capable of solubilizing potassium minerals quickly in large quantity can conserve our existing resources and avoid environmental pollution hazards caused by heavy application of chemical fertilizers.

Currently, very little information is available on mineral potassium solubilization by bacteria, their mechanisms of solubilization and their effect on growth, K uptake and yield of several crops.

Therefore the present investigation was undertaken with the following objectives.

1. Isolation and purification of mineral potassium solubilizing bacteria from rhizosphere soils of different crops
2. Characterization and *in vitro* screening of isolates for their efficiency
3. To assess the influence of selected efficient mineral potassium solublizing bacteria on growth, yield and K uptake of maize plants

2. REVIEW OF LITERATURE

Potassium is an essential major plant nutrient and also a non-renewable resource. It plays a vital role in plant metabolism such as photosynthesis, translocation of photosynthates, regulation of plant pores (stomata), activation of plant catalysts (enzymes) and imparts resistance in plant against pest and diseases. Without adequate potassium, the plants will have poorly developed roots, grow slowly, produce small seeds and have lower yields. They are also more susceptible to disease infection. Majority of the soils of the world are too low in available potassium for production of good yields. Certain groups of micro-organisms including bacteria, fungi and actinomycetes are known to soluiblize potassic minerals into soluble form which can be utilized by the plants. Since the literature pertaining to solubilization of potassic minerals by bacteria is very scanty. The available literature on potassium solubilization by bacteria and their mechanisms of solubilization, other beneficial traits and their agronomic importance is reviewed in this chapter.

2.1 POTASSIUM IN SOIL

Among the major plant nutrients potassium is the most abundant one in soils. It is also seventh most common element in the earth crust and on an average the surface layer (lithosphere) contain 2.5 per cent potassium. It exists mainly in three different forms such as soil minerals, available and non-exchangeable. Among three different forms of potassium in soil, the concentration of soluble K in soils is usually very low (1% to 2%) and the major proportion (98%) of K in soils is insoluble rocks and minerals (Goldstein, 1994).

The potassium content of Indian soils varies from less than 0.5 per cent to 3.00 per cent. The average total potassium content of these soils is 1.52 per cent (Mengel and Kirk, 1987). However, total K is rather poorly correlated with available K and is rarely used to describe K fertility status of a soil. The immediate source of K for plants is the small amount which is in the soil solution. The concentration of potassium in the soil solution is of the range from 1 to 2 per cent. As K is removed, the equilibrium is disturbed, K in the non-exchangeable and soil mineral fraction will be drawn upon. The supply of K to the plants depends directly on the concentration of K in soil solution and indirectly on soil, which maintains this equilibrium (Sparks and Huang, 1985).

Potassium content of Indian soils has traditionally been considered as adequate. In the recent years, however the importance of K and the need for its continuous optimal availability for the better crop production is felt as long before symptoms of K deficiency become visible severe losses in terms of yield and quality have been caused to crop and produce (Khanwilkar and Ramteke, 1993).

In mineral soils, K occur in the form of silicate minerals *viz.,* muscovite, orthoclase, biotite, feldspar, illite, mica, vermiculite, smectite *etc.* Total pool of soil K is extremely complex and this can be solubilized by bacteria through production of acids and it will be available for plant (Ullaman *et al*., 1996).

2.2 OCCURRENCE AND ISOLATION OF POTASSIUM SOLUBILIZATION BACTERIA (KSB)

Microbial inoculants that are able to dissolve potassium from mineral and rocks, have influence on plant growth and have both economic and environmental advantage. The first evidence of microbial involvement in soluiblization of rock potassium (Muntz, 1890).

Several microorganisms like *Aspergillus niger, Bacillus extroquens* and *Clostridium pasteurianum* were found to grown on muscovite, biotite, orthoclase microclase and micas in *in vitro* (Retimier, 1951).

A variety of soil microorganisms have been found to solubilize silicate minerals (Bunt and Rovira, 1955).

The microorganisms like bacteria, fungi and actinomycetes were colonized even on the surface of mountain rocks (Gromov, 1957), Norkina and Pumpyanskaya (1956) reported that the silicate solubilizing bacteria *B. mucilaginosus* subsp. *Siliceus* liberates potassium from feldspar and aluminosilicates. Duff and Webley (1959) reported silicate dissolving action of a gram negative bacteria *Erwinia, Bacterium herbicola* or with *Pseudomonas* strains.

Webley *et al.* (1963) demonstrated that the silicaceous materials in rocks can be attacked through the products of metabolism of microorganisms.

Alkasandrov *et al.* (1967) isolated different bacterial species like silicate bacteria were found to dissolve potassium, silica and aluminium from insoluble minerals. Heinen (1960) reported the ability of *Bacillus caldolyticus* and *Proteus* sp. to grow and soluiblize quartz

Purushothaman *et al.* (1974) reported the distribution of silicate solubilizing bacteria in marine environments and suggested that they play a role in cycling of silicon in sea water.

Belkanova (1985) reported the cleavage of siloxane bond in quartz by *B. mucilaginosus*. Among the K bearing silicate minerals mica was found to weather readily (Tandon and Sekhon, 1988).

Avakyan *et al.* (1981) and Belkanova (1985) reported *B. mucilaginosus* solubilize insoluble silicates, Webley *et al.* (1960) have reported that a *Pseudomonas* isolated from soil showed clearing zone in silicate medium similar results were shown by Purushothaman *et al.* (1974).

Avakyan *et al.* (1986) and Li *et al.* (1994) isolated K solublizing bacteria from soil, rock and mineral samples. The isolate MCRCP 1 was later identified as *B. mucilaginosus* based on morphological and physiological characters.

Muralikannan (1996) isolated silicate solubilizing bacteria from rice rhizosphere and tentatively identified as *Bacillus* sp.

Kannan and Raj (1998) carried out enumeration of silicate and phosphate solubilizing bacteria from soils tank sediments. Three out of 17 isolates were identified as *Bacillus* sp. based on biochemical characteristics. Lian (1998) isolated silicate bacteria a *B. mucilaginosus* from corn field.

Liu (2001) isolated silicate bacteria *B. mucilaginosus* CS 1 and CS 2 from soil and those were exhibited inhibitory activity on the growth of gram negative bacteria *E. coli* and they identified strain CS1 as *B. mucilaginosus*.

It has been reported that the ability of slime production the *B. mucilaginosus* dissolves the silicates and also colonises and develop in rhizosphere as well as non-rhizosphere soil (Lin *et al*., 2002).

Hutchens *et al.* (2003) studied twenty seven strains of heterotrophic bacteria from feldspar rich soil in liquid and solid minimal aerobic media and also studied silicate mineral dissolution.

Raj (2004) identified silicate soluiblizing bacteria from rice ecosystem (SSB) in a medium containing 0.25 per cent insoluble magnesium trisilicate and also reported that *Bacillus* sp. found to solubilize silicate minerals more efficiently under *in vitro* conditions.

Potassium soluiblizing rhizobacteria were isolated from the roots of cereal crop by the use of specific potassium bearing minerals (Mikhailouskaya and Tchernysh, 2005). Bardr (2006) reported a bacteria capable of dissolving silicate minerals from feld spar samples.

Hu *et al.* (2006) reported K soluiblizing strains from the soil and they were phenotypically and phylogenetically characterized and were effectively dissolve mineral potassium when they grown on Alekandrove medium which are rod shaped spore formers with a large capsule and formed slimy and translucent colonies.

Murali *et al.* (2005) isolated silicate soluiblizers using modified Bunt and Rovira medium from soil samples collected from coconut palms. Majority of the silicate solubilizers are identified as *Bacillus* sp. and *Pseudmonas* sp.

Zhou *et al.* (2006) characterized and identified as *Bacillus mucilaginosus* which soluiblizes silicon from illite at 30°C, the bacterium is identified as gram-negative, rod shaped with endospore former and thick capsule.

Sugumaran and Janarthanam (2007) isolated K solubilizing bacteria from soil, rocks and minerals samples *viz.,* orthoclase, muscovitemica. Among the isolates *B. mucilaginosus* solubilized more potassium by producing slime in muscovite mica.

2.3 MINERAL POTASSIUM SOLUIBLIZING POTENTIAL OF BACTERIA

Many of the indigenous soil microorganisms have the potential to absorb and mobilize the fixed form of nutrient from trace mineral sources.

Silicate bacteria were found to dissolve potassium, silica and aluminium from insoluble minerals and they are known to liberate phosphoric acids that solublize apatite and release available form of nutrients from apatite (Heinen, 1960).

Duff *et al*. (1963) isolated 2 keto-gluconic acid producing gram negative bacteria *Pseudomonas fluorescence* (Strain No. 2062) and reported that the isolated bacteria have the capacity to dissolve the resistant natural phosphate and silicates in the soil. Solubilization kinetics of phosphate released from the organic phosphate by the strain was about 17 per cent.

The efficiency of potassium soluiblization by different bacteria vary with a nature of potassium bearing minerals. Yakhontova *et al.* (1987) opined that the intensity of degradation of silicate minerals by the bacterium was dependent on the structure and chemical composition of the mineral and potassium dissolving ability of HM 8841 has been measured using Kietyote and Pegatolite 47 mg, 44.4 mg of soluble potassium released after 38 hours of incubation time.

Sheng *et al.* (2002) reported that potassium release from minerals affected by pH, dissolved oxygen and strain used. The content of potassium in solution inoculated with bacteria was increased by 84.8-127.9 per cent compared with the control. The extent of potassium solubilization by *B. edaphicus* in the liquid media and reported better growth on illite than feldspar (Sheng and He, 2006).

Sheng (2002) observed potassium release from strains of potassium solubilizing bacteria is 35.2 mg/l in 7 days at 28°C at pH range from 6.5-8.0.

Badr (2006) studied extent of potassium and phosphorus solubilization by silicate solubilizing bacteria it ranges from 490 mg/l to 758 mg/l at pH 6.5 to 8.0 and the potassium solubilization by *B. mucilaginosus* isolated from soil, rock and mineral samples recorded 4.29 mg/l release of potassium in media supplemented with muscovite mica (Sugumaran and Janarthanam, 2007).

The potassium releasing characteristics of a bacterium from different minerals were studied by using soil column experiment. Potassium release affected by pH, aerobic conditions and soil mineral properties more K was produced in a more aerobic condition than less aerobic. The release of potassium in order was illite > feldspar > muscovite (Sheng, 2002 and Badr, 2006).

2.4 MECHANISMS OF POTASSIUM SOLUBILIZATION

Moira *et al*. (1963) isolated many of the fungal isolates having the potential to release metal ions and silicate ions from minerals, rocks and soils. The minerals used in this study were saponite and vermiculite. These fungal isolates are known to produce citric acid, oxalic acid that are mainly known to decompose or solubilize natural silicates and help in removal of metal ions from the rocks and soils.

Jones and Handrecht (1967) reported that bacteria soluiblize the insoluble silicates by production of CO_2 organic acids and exopolysaccharides.

The solubilization of silicates was inestigated using Kaolin quartz and sand as model substances. The chemical leaching of silicates was carried out using inorganic and organic acids as well as sodium hydroxide. The process was more effective in the alkaline than in the acid pH range on the other hand, microbiological influence on solubilization. The transformation of crystalline biotite, mica, vermiculite and certain rocks to amorphous state is due to the action of some organic products of microbial metabolism (Weed *et al*., 1969).

Bacteria have also been shown to accelerate the dissolution of silicates by the production of excess proton and organic ligands and in some cases by the production of hydroxyl anion, extra cellular polysaccharides (EPS) and enzymes (Berthelin and Belgy,

1979; Malinovskaya *et al*., 1990; Hiebert and Bennett, 1992; Welch and Ullman, 1993; Vandevivere *et al*., 1994; Barker *et al*., 1998). Welch *et al.* (1999) found that a variety of extracellular polysaccharides significantly enhanced the dissolution of plagioclase at pH 4 but had little effect at pH 7.

Vainberg *et al.* (1980) proposed that dissolution of minerals was caused by the formation of organic acids in the culture media.

Berthelin (1983) reported potassium is soluiblized from precipitated forms through production of inorganic and organic acids by *Thiobacillus, Clostridium* and *Bacillus*. The mineral dissolution was enhanced due to production of mucilaginous capsules containing of exopolysaccharides (Groudev, 1987).

The production of organic acids such as acetate, citrate and oxalate by micro-organisms can increase mineral dissolution rate (Hazen *et al*., 1991 and Barker *et al*., 1998). Moreover results also suggest that the weathering ability of the bacteria which involves the production of protons, organic acids, siderophores and organic ligands (Grayston *et al*., 1996; Liermann *et al*., 2000; Paul and Clark, 1989; Rogers *et al*., 2004; Welch *et al*., 1999).

Argelis *et al.* (1993) reported weathering mechanisms carried out by *Pencillium frequentans* and *Cladosporium*, *Cladosporoides* on unaltered sand stone, granite and lime stone. They also reported that both fungal species isolated have the capacity to produce large amounts of oxalic, citric and gluconic acids in broth culture that cause extensive deterioration of clay silicates, mica and feldspar from both sand stone and granite and also of calcite and dolomite from lime stone. Finally they concluded that filamentous fungi able to cause an extensive weathering of stone due to organic acid excretion. The production of gluconate promotes dissolution of silicates like albite, quartz and koalinite by subsurface bacteria has been recorded Vandevivere *et al.* (1994).

Freidrich *et al.* (1991) and Ullman *et al.* (1996) reported (KSB) potassium soluiblizing bacteria *B. mucilaginosus* are able to soluiblize rock K mineral powder such as micas, illite and orthoclases through production and excretion of organic acids.

Palmer *et al.* (1991), Welch *et al.* (1993) and Styriakova *et al.* (2003) reported organic compounds produced by micro-organisms such as acetate, citrate and oxalate can increase mineral dissolution rates in laboratory experiments and in the soil by Sheng *et al.* (2003), Badar *et al.* (2006).

Welch and Ullman (1993) found that the rate of plagioclase dissolution in solutions containing organic acids were more compared to inorganic acids and showed that bacterium produces polysaccharides during the process of reproduction and these can combine with the minerals and form bacterial mineral complexes which leads to degradation of the minerals.

Styriakova *et al.* (2003) reported that the activity of siliate dissolving bacteria played a pronounced role in the release of Si, Fe and K from feldspar and Fe oxhydroxides.

Increasing evidence also exists for a mechanism of direct silicate precipitation by bacteria via metal sorption at the cell membrane (Bevridge and Murray, 1980; Beveridge and Fyfe, 1985; Ferris *et al*., 1989; Urruti and Beveridge, 1994; Konhauser and Ferris, 1996).

Microbially produced organic ligands include metabolic by products, extracellular enzymes, chelates and both simple and complex organic acids enhance the dissolution of aluminosilicate mineral or quartz both in field and laboratory epeirments (Grandstaff, 1986; Surdam and MacGowan, 1988; Huang and Longo, 1992; Welch and Ullman, 1993).

Sheng and Le (2006) reported that solubilization of illite and feldspar by microorganisms is due to the production of organic acids like oxalic acid and tartaric acids and also due to production of capsular polysaccharides which helps in dissolution of minerals to release potassium. Decomposition of silicate minerals by *B. mucilaginosus* due to production of oxalate and citrate and the extent of which polysaccharides absorbed organic acids decomposes minerals (Liu *et al*., 2006).

2.5 EFFECT OF INOCULATION OF POTASH SOLUBILIZERS ON PLANT GROWTH AND YIELD

The first report on increase in yield of maize and wheat by application of organo minerals inoculated with silicate bacteria (Alexandrov, 1958). The increase in yield of cotton by 50-94 per cent when Azotobacterin and silica bacterin were applied simultaneously (Ciobanu, 1961).

Vintikova (1964) observed the beneficial effects of silicate bacteria in Lucerne and maize.

Khudsen *et al.* (1982) and Krieg and Holt (1984) isolated potassium solubilizing bacteria from rock and mineral samples showed higher activity in potassium release from acid leached soil and improving greengram seedling growth. Phosphorus solubilizing bacteria and silicate bacteria play a important role in plant nutrition through the increase in P and K uptake by plant (Datta *et al*., 1982 and Nianikoval *et al*., 2002).

Zahro *et al.* (1984) studied the effect of soil inoculation of the silicate bacteria.*Bacillus circulans* on the release of K and Si from different minerals and in different soil resulted that the bacteria could persist for a long time where high population density could be detected after 14 month particularly in soils containing higher levels of organic matter. An increased yield in rice crop was observed due to inoculation of silicate solubilizing bacteria (Muralikannan, 1996 and Kalaiselvi, 1999).

Xue *et al.* (2000) and Sheng *et al.* (2003) reported silicate dissolving bacteria could improves soil P, K, Si reserves and promote plant growth. The effect of potash mobilizer on brinjal has recorded an increased potash uptake and increased plant biomass in potash mobilizer treated plants as compared to the control plants (Nayak, 2001).

Lin *et al.* (2002) recorded increase in biomass by 125 per cent K and P uptake were more than 150 per cent in tomato plant due to inoculation of silicate dissolving bacteria (*B. mucilaginosus*) than the non-inoculation. Thus there is a potential in applying RCBC13 for improving K and P nutrition. Effect of plant growth promoting rhizobacteria (PGPR) including phosphate and potassium soluiblizing bacteria (PSB and KSB) as biofertilizers as a sustainable solution to improve plant nutrient and production (Vessey, 2003).

Park *et al.* (2003) reported that bacterial inoculation could improve phosphorus and potassium availability in the soils by producing organic acid and other chemicals by stimulating growth and mineral uptake of plants.

Sheng *et al.* (2003) studied the effect of inoculation of SSB (*Bacillus edaphicus*) on chilli and cotton which resulted in increased in available P and K contents in plant biomass.

In a study to assess the weathering of finely ground phogopite a trictahedral mica by placing it in contact with heterotrophic bacteria *B. cereus* and acidophilic (*Acidothiobacillus ferroxidans*) cultures enhanced the chemical dissolution of the mineral. The x-ray diffraction analysis of the phologopite sample before and after 24 weeks of contact with *B. cereus* cultures revealed a decrease in the characteristic peak intensities of phologopite indicating distruction of individual structural planes of the mica on the other hand, *Acidothiobacillus ferroxidans* cultures enhanced the chemical dissolution of the mineral and formed partially weathered interlayer from where K was expelled. This was coupled with the precipitation of K and Jarosite (Styriakova *et al*., 2004).

Zhang *et al.* (2004) reported that the effect of potassic bacteria on sorghum, which results in increased biomass and contents of P and K in plants than the control. The effect of SSB (*Bacillus* sp.) on grain yield and plant silica content of rice and available silica in soil (Raj, 2004). The increased K uptake coupled with increased yield in yam and tapioca while treating the plants with potassium moiblizer in conjunction with biofertilizers and chemical fertilizers (Clarson, 2004).

Chandra *et al.* (2005) reported that increased yield by 15 to 20 per cent in yam and tapioca due to the potash solubilizer application and in combination with other biofertilizer like *Rhizobium, Azospirillum, Azotobacter, Acetobacter* and PSM.

Wu *et al.* (2005) found inoculation of K solubilizer (*B. mucilaginosus*) along with P soluiblizer (*B. megaterium*) and N-fixer (*Azotobacter chroococcum*) increased the growth, nutrient uptake significantly in maize crop and also improved soil properties such as organic matter content and total N in soil.

Han and Lee (2005) found that the co-inoculation of PSB and KSB in combination with direct application of rock P and K materials into the soil resulted increased N, P and K uptake, photosynthesis and the yield of egg plant grown on P and K limited soil.

Ramarethinam and Chandra (2005) in a field experiment recorded increased brinjal yield, plant height and K uptake significantly compared to control due to inoculation of potash solubilizing bacteria (*Frateuria aurantia*).

Mikhailouskaya and Tchernysh (2005) reported on effect of inoculation of K mobilizing bacteria on severally eroded soil which are comparable with yields on moderately eroded soil with out bacterial inoculation resulted increased wheat yield upto 1.04 t/ha.

Sheng (2005) worked on potassium releasing bacterial strain *B. edaphicus* for plant-growth promoting effects and nutrient uptake on cotton and rape seed in K-deficient soil pot experiments resulted increased root and shoot growth and potassium content was increased by 30 and 26 per cent respectively. And in chilli crop increased biomass and K uptake due to inoculation of potash soluiblizer (Ramarethinam *et al*., 2005).

Christophe *et al.* (2006) reported that *Burechulderia glathei* in association with pine roots significantly increased weathering of biotite and concluded that there was the effect of *B. glathei* PMB (7) and PML1 (12) on pine growth on root morphology which was attributed to release of K from the mineral.

Sheng and He (2006) recorded an increased root and shoot growth and also showed significantly higher N, P and K contents of wheat plants components due to inoculation of *B. edaphicus* growth in a yellow brown soil that had low available K. And in the field experiment recorded increased yield in tomato crop due to inoculation of silicate dissolving bacteria *B. cereus* as bioinoculant along with feldspar and rice straw on K releasing capacity (Badr, 2006).

Han *et al*. (2006) evaluated the potential of PSB and KSB inoculated in nutrient limited soil planted with pepper and cucumber results showed that coinoculation of PSB and KSB showed high P and K content and plant growth compare to control.

Supanjani *et al*. (2006) reported that integration of P and K rocks with inoculation of phosphorus and potassium solubilizing bacteria increased P availability from 12 to 21 per cent and K availability from 13 to 15 per cent. In the soil as compare with control and subsequently improved nutrient N, P and K uptake in *Capsicum annuum*. The integration also increased plant photosynthesis by 16 per cent and leaf area by 35 per cent as compare to control on the other hand the biomass harvest and fruit yield of the treated plants were increased by 23 per cent to 30 per cent respectively over all results of this finding is the treatment with P and K rocks and P and K solubilizing bacterial strain were sustainable alternative to the use of chemical fertilizer.

Badar *et al.* (2006) studied on effect of bacterial inoculation combined with K and P bearing minerals on sorghum plants and reported increased in dry matter yield and P and K uptake in three different soils clay sandy and calcareous soils, 48, 65 and 58 per cent increase in dry matter, 71, 110 and 116 per cent uptake of P and 41, 93 and 79 per cent uptake of K and improved fertility through inoculation of SDB. The increased rice grain yield in a field experiment due to effect of silicate solubilizing bacteria recorded 5218 kg/ha grain yield than control 4419 kg/ha (Balasubramanian and Subramanian, 2006).

The potential phosphate solubilizing bacteria (PSB) *B. megaterium* var. *Phosphaticum* and potassium soluiblizing bacteria (KSB), *B. mucilaginosus* were evaluated using pepper and cucumber as test crops. The out come of the experiment showed that rock phosphorus and potassium applied either singly or in combination donot significantly enhance availability of soil phosphorus and potassium indicating their unsuitability for direct application co-incubation of PSB and KSB resulted in consistently higher P and K available than in the control (Vassilev *et al*., 2006).

Sugumaran and Janarthanam (2007) recorded increase in the dry matter by 125 per cent and oil content 35.4 per cent of groundnut plant and available P and K is increased from 6.24 and 9.28 mg/kg and 86.57 to 99.60 mg/kg respectively in soil due to inoculation of *B. mucilaginosus* (KSB) compared to uninoculated control.

3. MATERIAL AND METHODS

The present investigation was carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad on isolation and characterization of potassium soluiblizing bacteria obtained from rhizosphere soil of different crops. The bacterial cultures were tentatively identified upto genus level based on their morphological and biochemical characteristics. The selected efficient isolates were further examined for their ability to solubilize potassium from insoluble K source and for other beneficial traits and were also tested for their potential in enhancing the growth and yield of maize plants under pot culture studies. The materials used in the study, the procedures and the techniques which were adopted are detailed in this chapter.

3.1 ISOLATION OF POTASSIUM SOLUBILIZING BACTERIA

3.1.1 Collection of soil samples

The rhizosphere soils of different crops plants were collected in the areas of Dharwad and Belgaum districts. The samples were brought in polythene bags.

3.1.2 Isolation and purification of potassium soluiblizers

Potassium solubilizing bacteria were isolated from collected soil samples by serial dilution plate count method using Aleksandrov medium (Hu *et al*., 2006) which is a selective medium for isolation of potassium solubilizers. Agar medium containing insoluble potassium bearing mineral (mica). The plates were incubated at room temperature (30±1°C) for 3 days and the colonies exhibiting clear zones were selected, purified by four way streak plate method. The diameter of zone of solubilization was measured and expressed in centimeter and the selected isolates were preserved on agar slants for further use.

3.2 IDENTIFICATION AND CHARACTERIZATION OF THE BACTERIAL ISOLATES

All the selected isolates were examined for the colony morphology, cell shape, gram reaction and ability to form spores as per the standard procedures given by Anonymous (1957) and Barthalomew and Mittewer (1950).

3.2.1 Biochemical characterization

The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992). The tests conducted are detailed below.

3.2.1.1 Starch hydrolysis

The ability of the isolates to hydrolyse starch was examined by the procedure of Eckford (1927). Petriplates containing starch agar were inoculated with test cultures and incubated at 30°C for three days. After incubation the plates were flooded with Lugol's idodine solution and allowed to stand for 15-20 minutes. The clear zone around the colony was considered as positive for the test.

3.2.1.2 Casein hydrolysis (Seeley and Vandemark, 1970)

Plates containing skim milk agar was streaked with test cultures and incubated at 30°C for one week. The clear zones around the colony against a black background after incubation was taken as positive for casein hydrolysis.

3.2.1.3 Acid and gas production (Seeley and Vandemark, 1970)

The isolates were tested for acid and gas production by inoculating five ml of pre-sterilized glucose broth medium in test tubes containing Durham's tube and bromocresol purple (15 ml/l of 0.04% solution) as pH indicator. The tubes were incubated for seven days at 30°C. The accumulation of gas in the Durham's tube was taken as positive for gas production and the change in colour of medium from purple to yellow was taken as positive for acid production.

3.2.1.4 Catalase test (Blazevic and Ederer, 1975)

Nutrient agar slants were inoculated with test organisms and were incubated at 30°C for 24 hours. After incubation the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for production of gas bubbles. The occurrence of gas bubbles was scored positive for catalase activity.

3.2.1.5 Hydrogen sulphide production (Cowan and Steel, 1970)

Bacterial isolates were inoculated to test tubes containing 5 ml of sterile medium and incubated at room temperature 28°C. The test tubes were observed for H_2S production. The formation of black ring in the medium was taken as positive for H_2S production.

3.2.1.6 Urease test (James and Natalie Sherman, 1992)

Bacterial isolates were tested for urease activity by inoculating the cultures to five ml of pre-sterilized urea broth containing phenol red as pH indicator. The tubes were incubated for 24 to 48 hours at 30°C. The formation of dark pink colour was taken as positive for urease activity.

3.2.1.7 Gelatin liquefaction (Blazevic and Ederer, 1975)

To the pre sterilized nutrient gelation deep tubes, the test cultures were inoculated and tubes were incubated at 28±2°C for 24 hours. Following this, the tubes were kept in a refrigerator at 4° for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test.

3.2.1.8 Oxidase test (Cappuccino and Sherman, 1996)

To the trypticase soyagar plates, overnight culture of the test isolate was spotted and the plates were incubated for 24 hours at 28±2°C. After incubation, two to three drops of tetramethyl phenylenediamine dihydrochloride was added to the surface of the growth of test organism. The colour change to maroon was taken as oxidase positive.

3.2.1.9 Denitrification test

Nitrate broth tubes with inverted Durham's tube inside were inoculated with the overnight grown culture of the test organism and that were incubated for two weeks at 25°C. After one week of incubation the inverted Durham's tube were observed for the accumulation of gas.

3.2.1.10 Methyl red test (Seeley and Vandemark, 1981)

Test culture containing MR-VP broth were sterilized and inoculated with the test cultures. The tubes were incubated at 28±2°C for 48 hours. After incubation five drops of methyl red indicator was added to each tube and gently shaken. The production of red colour was taken as positive for the test and production of yellow colour was taken as negative for the test.

3.2.1.11 Voger – Proskauer test (Seeley and Vandemark, 1981)

To the pre-sterilized tubes containing MR-VP broth test cultures were inoculated. The tubes were incubated for 48 hours at 37°C. After incubation tendrops of Barritt's reagent A was added and gently shaken followed by addition of ten drops of Baritt's reagent B. The development of rose colour in the broth was taken as positive for the test.

3.2.1.12 Growth at 7 per cent NaCl

The tubes of nutrient broth 3 ml/tube containing seven per cent sodium chloride were inoculated with a loop full culture of the test isolates grown overnight in nutrient broth. The tubes were incubated at 28±2°C and the growth was observed after 24 hrs in terms of turbidity which was taken as positive for the test.

3.2.1.13 Utilization of different carbon sources

The isolates were examined for their ability to utilize different carbon sources *viz.,* sucrose, maltose, glycerol and citrate. The carbon sources were added at the concentration of two per cent to the agar medium and 24 hour old cultures were streaked on the surface of agar medium and incubated at 28±2°C for 24 hours. The extent of growth on the media

containing different carbon sources was observed usually and growth was scored as no growth (-) and growth (+).

3.3 SCREENING OF ISOLATES FOR MINERAL POTASSIUM SOLUBILIZATION

3.3.1 Quantitative estimation of K released from insoluble K bearing mineral

The isolates showing zone of solubilziation on Aleksandrov agar were further examined for their ability to release K from broth media (supplemented with 1 per cent muscovite mica). One ml of overnight culture of each isolate was inoculated to 25 ml of Aleksandrov broth (Hu *et al*., 2006) in nine replicates. All the inoculated flasks were incubated for two week at 28±2°C. The amount of K released in the broth was estimated at 7, 15 and 20 days of incubation from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 minutes in the remi microcentrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry (Sugumaran and Janarthanam, 2007).

One ml of the culture supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with distilled water and mixed thoroughly. After that the solution was fed to flame photometer and K content was determined.

Simultaneously, a standard curve was prepared using various concentrations of 2 ppm KCl solution. The amount of potassium solubilized by the isolates was calculated from the standard curve.

3.3.2 Preparation of standard curve

Potassium chloride was dried at 60°C and 1.908 g of it was dissolved in distilled water and made up the volume to one liter. Ten ml of this was diluted furter to 100 ml with distilled water to obtain 2 ppm solution and used for preparation of standards 0, 2, 4, 6, 8, 10 ppm. These standards were fed to flame photometer to obtain K standard curve.

3.3.3 Organic acid production by K-solubilizing isolates

One ml of 24 hours old culture of each isolate was inoculated to 25 ml of Aleksandrov broth and incubated at 28±2°C for 10 days. The broth culture was centrifuged at 10,000 rpm for 10 minutes. The supernatant so obtained was concentrated to nearly $1/10^{th}$ of the original volume in a water bath at 60°C. The concentrated material was then used for determination of organic acid by paper chromatography in comparison with standard organic acids (Gaur, 1990).

Pure organic acids were prepared at 20 g/ml stock. About 10 µl of these standard acids and 15 µl of culture supernatants were spotted on Whatman No. 1 chromatographic paper and dried with a hair dryer. A descending chromatography was run using a solvent mixture of n-butanol acetic acid and water in 12:3:5 ratio in a chromatographic chamber presaturated with solvent for six hours.

The chromatogram was run for 6 hours and air dried for three days. The air dried paper was sprayed with 0.04 per cent bromocresol green (40 g BCG in 1000 ml methanol pH 7.0).

The paper was air dried at room temperature. The Rf values and the intensity of yellow spots of organic acids developed on a blue background were measured and compared with the Rf values of the standard organic acids for identification.

3.4 OTHER BENEFICIAL TRAITS

The isolates were examined for other beneficial traits *viz.,* solubilization of insoluble inorganic phosphate and production of plant growth promoting substances like IAA and GA.

3.4.1 Phosphate solubilizing ability of the isolates

All the bacterial isolates were tested for their ability to solubilize insoluble inorganic phosphate on Pikovskaya's agar plates by the procedure suggested by Pikovskaya (1948).

3.4.2 Polysaccharide production

All the efficient K solubilizing bacteria were tested for polysaccharide production by spotting 10 µl of overnight culture on glucose minimal agar medium (Sambrook *et al*., 1989). The plates were incubated at 28±2°C for 24 to 48 h. The amount of polysaccharide produced on glucose minimal agar medium was observed visually and scored as no polysaccharide production (-), weak polysaccharide production (+), moderate polysaccharide production (++) and high polysaccharide production (+++) by the strains.

3.4.3 Production of growth promoting substances by the isolates

All the isolates were subjected to qualitative analysis for the production of IAA (Bric *et al*., 1991) and GA (Brown and Burlingham, 1968).

Luria agar supplemented with 0.06 per cent sodium dodecyl sulphate and one per cent glycerol was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm x 2 cm by marking on the bottom of each plate. The overnight culture of each isolate grown on luria agar was spotted with sterile tooth pick in each square. The spotted plates were overlaid immediately with sterile disc of Whatman No. 1 filter paper. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. After an appropriate incubation period, the filter paper discs were removed from the plates and treated with Salkowaski's reagent (2% of 0.5 M $FeCl_3$ in 35% perchloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed until adequate colour was developed.

Bacteria producing IAA were identified by the formation of characteristic red halo around the colony on filter paper. The paper discs after treatment with Salkowaski's reagent were viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production.

The isolates showing IAA and GA production were further examined for the amount of IAA and GA production as detailed below.

3.4.4 Quantitative estimation of IAA and GA extraction

The overnight cultures of the isolates which showed the production of IAA and GA in quantitative estimation were inoculated to 50 ml of sterilized Czapeck's solution and incubated at 37°C for seven day in dark. After incubation, the cultures were centrifuged at 6000 rpm for 20 minutes. The supernatant was collected in a conical flask and used for estimation of IAA and GA.

3.4.4.1 Estimation of IAA (Gordon and Paleg, 1957)

Twenty five ml of the supernatant was collected and the pH was adjusted to 2.8 using 1N HCl in a 100 ml conical flask. Equal volume of diethyl ether was added to it and incubated in dark for four hours. Extraction of IAA was done at 4°C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gordon and Paleg (1957).

To 0.5 ml of methanol, 1.5 ml of distilled water and four ml Sapler's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour develop was read at 535 nm in a UV-visible spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as µg/25 ml of the medium.

3.4.4.2 Estimation of GA (Paleg, 1965)

Twenty five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes, two ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant was added five ml of 30 per cent HCl and incubated at 20°C for 75 minutes. The blank sample was treated with five per cent HCl and the absorbance of the samples as well as blank was measured at 254 nm in a UV-visible spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as µg/25 ml of the medium. The standard curves of IAA and GA were prepared by using graded concentrations of IAA and GA_3.

3.4.5 Biocontrol potential

The bacterial isolates were tested for their *in vitro* antipathogenic activity against *Macrophomina phaseoli* on potato dextrose agar (Appendix I) plates by dual inoculation technique (Saktivel *et al*., 1986).

The fungal pathogen was grown on PDA plate for 72 hours. With the help of sterile cork borer, the disc of fungal growth from this plate was taken out and placed at the centre of the fresh PDA plate. Ovenright growth of each bacterial isolate was then streaked parallelly on either side of the disc and kept for incubation at 30℃ for 72 hours. Visual observations were recorded after 72 hours of incubation for the inhibition of fungal pathogen by comparing with the PDA plate inoculated with only fungal pathogen.

3.5 GREEN HOUSE EVALUATION OF EFFICIENT POTASSIUM SOLUBILIZING BACTERIA (KSB) FOR GROWTH NUTRIENT UPTAKE AND YIELD OF MAIZE PLANTS

A pot culture experiment was conducted using nine efficient potassium solubilizing bacteria in comparison with reference strain obtained from liquid biofertilizer, Regional Biofertilizers Development Centre, Bangalore, to study their performance in enhancing the growth K uptake and yield of maize plants as detailed below.

3.5.1 Treatments

The treatments fixed for pot culture experiment presented in Table 3.1. Fourteen treatments, each with five replications were designed, two replications were used to record observation on plant growth parameters after 55 days of plant growth and three were used to record observation on dry biomass and yield at harvest.

Table 3.1 Details of the treatments used for pot culture experiment

Sl. No.	Treatments
1	Absolute control (No inoculation, no fertilizer K)
2	Potassium control 1 (25% RDK)
3	Potassium control 2 (75% RDK)
4	Potassium control 3 (100% RDK)
5	*Frateuria aurantia* (Reference strain)
6	Isolate KSB 11
7	Isolate KSB 14
8	Isolate KSB 16
9	Isolate KSB 18
10	Isolate KSB 33
11	Isolate KSB 35
12	Isolate KSB 42
13	Isolate KSB 47
14	Isolate KSB 62

N and P were applied to all the treatments at recommended dose of fertilizers

Recommended dose of fertilizer to maize is 150:75:37.5 kg $N:P_2O_5:K_2O$ ha^{-1} respectively,

N – Urea
P – Single super phosphate
K – Muriate of potash

Total number of treatments : 14
Number of replication : 5
Design : CRD

3.5.2 Soil type

The medium deep black soil collected from E block of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad was sieved and thoroughly mixed with sand in 10:1 proportion and filled in the earthen pots of 30 cm diameter at the rate of 18 kg/pot. The required quantity of FYM (150 g/pot) were weighed separately for each pot and incorporated into the soil.

3.5.3 Soil characteristics

The soil was analyzed for its available nitrogen content by Kjeldhal method (Subbaiah and Asija, 1966) and organic carbon content by wet oxidation method (Jackson, 1967). The pH of the soil was determined in 1:2.5 soil solution using a digital pH meter. The available phosphorus content was determined by Olsen's method (Muhr *et al.*, 1965) and the available potassium by flamephotometer method (Stanford and English, 1949). The properties of soil are presented in Table 3.2.

Table 3.2 Characterization of the soil used

1. Morphological properties of soil		
a. Bacteria	8.1 x 10^6 cfu/g soil	
b. Fungi	4.3 x 10^3 cfu/g soil	
c. Actinomycetes	2.2 x 10^3 cfu/g soil	
2. Chemical properties of soil		
a. Electrical conductivity (dS/m)	0.17	EC bridge (Jackson, 1973)
b. pH	7.5	pH meter (Jackson, 1967)
c. Organic carbon (%)	0.43	Walkely and Black's wet oxidation method (Jackson, 1967)
d. Available nitrogen (kg/ha)	231.3	Alkaline permanganate method (Subbaiah and Asija, 1966)
e. Available P_2O_5 (kg/ha)	32.5	Olsen's method (Muhr *et al.*, 1965)
f. Available K_2O (kg/ha)	130.0	Flame photometer method (Stanford and English, 1949)

3.5.4 Seed

Maize (*Zea mays* L.) seeds of hybrid DMH-2 obtained from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad were used in the trial.

3.5.5 Fertilizers

The recommended dose of fertilizer used for maize (150:75:37.5 kg NPK per hectare) P was applied in the form of single super phosphate and N in the form of urea, while potassium was applied in the form of muriate of potash were added added according to the treatment schedule.

3.5.6 Seed treatment

Maize seeds were inoculated following the method of (Weller and Cook, 1983). The strains selected for the treatments were grown on medium for 48 hours. Growth was scraped and thoroughly mixed with one per cent sterile corboxy methyl cellulose (CMS) suspension. Maize seeds were surface sterilized with sodium hypochlorite (4%) for 25 minutes and then thoroughly rinsed twice with sterile water. The seeds were then placed in CMC based culture suspension and air dried overnight by placing in a laminar air flow chamber.

3.5.7 Sowing and maintenance

The inoculated seeds were sown in pots @ 4 seeds per pot in five replications. After germination, thinning was done to retain only one plant in each pot. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pests and diseases.

3.5.8 Observations

Observations on plant growth parameters were recorded at 55 days after sowing (DAS) using three replications where as growth, yield and yield parameters were recorded at harvest (10 days) using the other three replicate pots.

3.5.9 Plant growth parameters

Plant height

The plant height was measured at 55 DAS from the base of the plant to the base of fully opened top leaf and expressed in centimeters (cm).

Number of leaves per plant

Number of leaves per plant were counted and recorded at 55 days of plant growth.

Girth of the stem

The girth of the stem was measured at 55 DAS by measuring circumference measured at the centre of the plant in cm was taken as girth of the stem.

Root growth

Root length was recorded at 55 DAS by uprooting the plants and measuring the root length from tip of the longest root to the neck region and expressed in cm.

Dry matter content

The dry matter content of maize plants was recorded at 55 DAS. From uprooted plants, the root and shoot portions were separated and air dried separately in an oven at 60℃ till constant weight was obtained. The shoot and root dry weight were recorded and expressed in g/plant.

3.5.10 Yield parameters

Straw weight (g/plant)

The straw weight after harvesting of each plant at physiological maturity was recorded for each treatment after air drying.

Cob weight (g)

The weight of cob from each plant in each treatment was recorded and expressed as g/plant.

Grain weight (g)

The grains from the cob of each plant were separated manually, grain weight was recorded for each treatment and expressed as g/plant.

Hundred seed weight

The weight of 100 grains randomly selected from each treatment was recorded and expressed in grams.

3.6 CHEMICAL ANALYSIS OF PLANT

3.6.1 Estimation of chlorophyll

The total chlorophyll content was determined by using dimethyl sulfoxide (DMSO) method given by Shoef and Lium (1976).

Fresh leaf samples (10 mg) were incubated in 7.0 ml of DMSO at 65°C for 50 minutes. At the end of the incubation period decanted the supernatant and discarded the leaf tissue. Made up the volume of supernatant to 10 ml with DMSO. Read the absorbance of extract at 645 and 663 nm using DMSO as blank.

$$\text{Total chlorophyll} = 20.0\ (A_{645}) + 8.0\ (A_{663}) \times \frac{V}{100 \times w \times a}$$

Where,

A = Absorbance at specific wave length (645 and 663 nm)

V = Final volume of chlorophyll extract (ml)

W = Fresh weight of sample (g)

a = path length of light (1 cm)

3.6.2 Nutrient uptake studies

The oven dried plant samples were ground to fine powder and used for estimation of potassium content.

Digestion of plant samples (wet oxidation)

One gram of powdered plant samples were pre-digested with concentrated nitric acid for overnight. Pre-digested samples were treated with diacid mixture (HNO_3:$HClO_4$ in 10:4 ratio) and kept on sand bath for digestion. After complete digestion, the precipitate was dissolved in 6 N HCl and transferred to the 100 ml volumetric flask and filtered through Whatman No. 42 filter paper, washed thoroughly with double distilled water and finally made the volume to 100 ml with double distilled water and preserved for further analysis.

3.6.3 Potassium uptake studies

3.6.3.1 Potassium uptake (mg/plant)

Potassium content of shoot and root was estimated (Stanford and Engligh, 1949) at 55 DAS and at harvest. Potassium uptake was calculated and expressed as mg per plant.

3.7 STATISTICAL ANALYSIS OF THE DATA

The data obtained from the experiments were subjected to statistical analysis by Completely Randomized Design. Interpretation of the data was carried out in accordance with Panse and Sukhatme (1985). The level of significance used in the F and ‘t’ test was P=0.01. The critical difference values were calculated wherever the ‘f’ test values were significant.

4. EXPERIMENTAL RESULTS

In this study attempts were made to isolate potassium solubilizing bacteria from rhizosphere soil of different crop plants around Dharwad and Belgaum districts. The isolates were examined for their ability to solubilize insoluble potassic mineral. The selected isolates were characterized and tentatively identified upto genus level based on morphological and biochemical properties. The efficient K soluiblizers were further subjected for their ability to release K from potassic mineral, mechanisms involved in K solubilization, phosphorus solubilization from TCP, production of plant growth promoting substance and for other beneficial traits. Highly efficient K solubilizing strains were also tested for their influence on growth and nutrient uptake of maize plant under pot culture conditions. The results obtained in these studies are presented here under.

4.1 ISOLATION OF POTASSIUM SOLUIBLIZING BACTERIA (KSB) FROM RHIZOSPHERE SOILS OF DIFFERENT CROPS

The rhizosphere soil samples of different crops were collected and used for the isolation of KSB. The details of the place of soil sample collected and the crops from whose rhizosphere the KSB were isolated are furnished in Table 4.1. Out of them total 30 KSB isolates selected 22 were from Belgaum and 8 from Dharwad district from rhizosphere of sorghum, maize, bajra, paddy, chilli, cotton, tomato, soybean, groundnut and banana. These isolates were purified, identified and maintained for further use.

4.2 IDENTIFICATION OF KSB ISOLATES

All the selected isolates of KSB were identified upto genus level based on their morphological and biochemical characters and the results are presented in Table 4.2. Among 30 isolates 26 were gram positive rods belongs to genera *Bacillus*. But four isolates were gram negative rod belongs to genera *Pseudomonas*.

4.3 POTASSIUM SOLUIBLIZATION BY THE ISOLATES

Qualitative analysis of the isolates for K solubilization are presented in Table 4.3. All the isolates examined for their ability to solubilize muscovite mica on agar media supplemented with mica at 1 per cent.

The diameter of zone of solubilization formed by the isolates ranged from 0.68 to 1.30 cm at 72 hours after incubation HAI. Among the isolates KSB11, KSB42 and KSB62 recorded maximum soluiblization zone (1.30 cm diameter) followed by KSB 16 (1.20 cm) and KSB14, KSB18, KSB33, KSB35 and KSB47 (1.00 cm). However, the isolate KSB25 showed the least solubilization zone of 0.68 cm diameter.

4.4 QUANTITATIVE ESTIMATION OF K SOLUBILIZING ACTIVITY OF THE ISOLATES

The amount of K released from muscovite mica in a broth by the isolates was studied at 7, 15, 20 days after incubation (DAI). The results are (Table 4.4) indicated that the amount of K released from mineral K by all strains increased with increase in incubation time and was maximum at 20 DAI. The K released from muscovite mica by the strain at 20 DAI ranged from 2.41 µg/ml to 44.49 µg/ml.

Among the isolates KSB11 released maximum amount of K from mica 44.49 µg/ml followed by KSB 42 37.07 µg/ml, both were significantly superior over all other isolates. But were on par among themselves. Out of 30 isolates examined nine isolates showed more than 20 µg/ml of potassium solubilization from muscovite mica.

Table 4.1 Details of places and crop plants used for isolation of potassium solubilizing bacteria

Sl. No.	Place	District	Crop	Isolates obtained
1	Kittur	Belgaum	Banana	KSB 1, KSB 67, KSB 69, KSB 3
2	Kittur	Belgaum	Chilli	KSB 4
3	Kuluvalli	Belgaum	Maize	KSB 5
4	Chikkodi	Belgaum	Sorghum	KSB 7
5	Gokak	Belgaum	Banana	KSB 10, KSB 64, KSB 35
6	Athani	Belgaum	Sorghum	KSB 11
7	Herikumbi	Dharwad	Soyabean	KSB 14
8	Saundatti	Belgaum	Banana	KSB 15, KSB 16
9	Dyampur	Dharwad	Maize	KSB 18
10	Ghataprabha	Belgaum	Banana	KSB 21, KSB 62
11	Chikkodi	Belgaum	Maize	KSB 22
12	Kagawad	Belgaum	Tomato	KSB 23
13	Agadi	Dharwad	Sorghum	KSB 25
14	Kurahatti	Dharwad	Groundnut	KSB 27
15	Annigeri	Dharwad	Maize	KSB 29
16	Kusugal	Dharwad	Maize	KSB 32
17	Saundatti	Belgaum	Rice	KSB 33
18	Yaragatti	Belgaum	Cotton	KSB 42
19	Adargunchi	Dharwad	Bajra	KSB 45
20	Kusugal	Dharwad	Rice	KSB 47
21	GTC	Dharwad	Soybean	KSB 48
22	Hukkeri	Belgaum	Sorghum	KSB 50
23	Nippani	Belgaum	Sorghum	KSB 55

Table 4.2 Morphological and biochemical characteristics of the potassium solubilizers

Sl. No	Isolates	Morphological characters		Spore formation	Biochemical test													Carbon source utilization				Probable genus
		Colony characters	Gram reaction & cell shape		1	2	3	4	5	6	7	8	9	10	11	12	13	a	b	c	d	
1	KSB 1	White, smooth, slimy, widely, spreading	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
2	KSB 3	Grayish white, smooth, widely spreading	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
3	KSB 4	Lave smooth, opaque creamy white flat surface	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
4	KSB 5	White, raised circular	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
5	KSB 7	Whitish, rough transparent	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
6	KSB 10	White, smooth widely spreading	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
7	KSB 11	Creamy white, smooth widely spreading, large size	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
8	KSB 14	Creamy white small	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
9	KSB 15	Creamy white irregular, opaque	-ve, rod	-	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	*Pseudomonas*
10	KSB 16	White, rough surface round	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
11	KSB 18	Creamy, small round spreading	-ve, rod	-	-	-	+	+	-	+	-	-	-	-	-	+	-	+	+	+	-	*Bacillus*
12	KSB 21	Creamy white smooth, small raised	-ve, rod	-	-	-	+	+	-	+	-	-	-	-	-	+	-	+	+	-	-	*Pseudomonas*
13	KSB 22	Creamy white smooth, small raised	+ve, rod	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Pseudomonas*
14	KSB 23	White, smooth circular, opaque	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
15	KSB 25	White smooth slimy large	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*

Contd…..

Sl. No	Isolates	Morphological characters		Spore formation	Biochemical test													Carbon source utilization				Probable genus
		Colony characters	Gram reaction & cell shape		1	2	3	4	5	6	7	8	9	10	11	12	13	a	b	c	d	
16	KSB 27	White smooth slimy large	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
17	KSB 29	Greyish white smooth widely spreading	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
18	KSB 32	Large smooth opaque creamy white	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
19	KSB 33	Large, smooth, opaque, creamy white	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
20	KSB 35	Creamy, smooth, raised, large	-ve, rod	-	-	-	+	-	-	+	-	-	-	-	+	+	-	+	+	-	-	*Pseudomonas*
21	KSB 42	White raised slimy	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
22	KSB 45	White raised slimy, large	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
23	KSB 47	Creamy white small, slimy	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
24	KSB 48	White, raised, circular	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
25	KSB 50	Creamy white, smooth, large	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
26	KSB 55	Creamy white, raised, irregular	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
27	KSB 62	Creamy white small	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
28	KSB 64	Creamy white large, circular	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
29	KSB 67	White, circular slimy	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*
30	KSB 69	Whit circular slimy, round	+ve, rod	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	-	-	*Bacillus*

1 – Starch hydrolysis, 2 – Casein hydrolysis, 3 – Urea hydrolysis, 4 – Gelatin liquefaction, 5 – Catalase test, 6 – Acid production, 7 – Nitrate reduction test, 8 – Methyle red test,
9 – Growth at 7% NaCl, 10 – H_2S production, 11 – Gas production, 12 – Citrate utilization, 13 – V. P. text, a – Mannitol, b – Sucrose, c – Maltose, d – Citric acid
+ : Positive, - : Negative

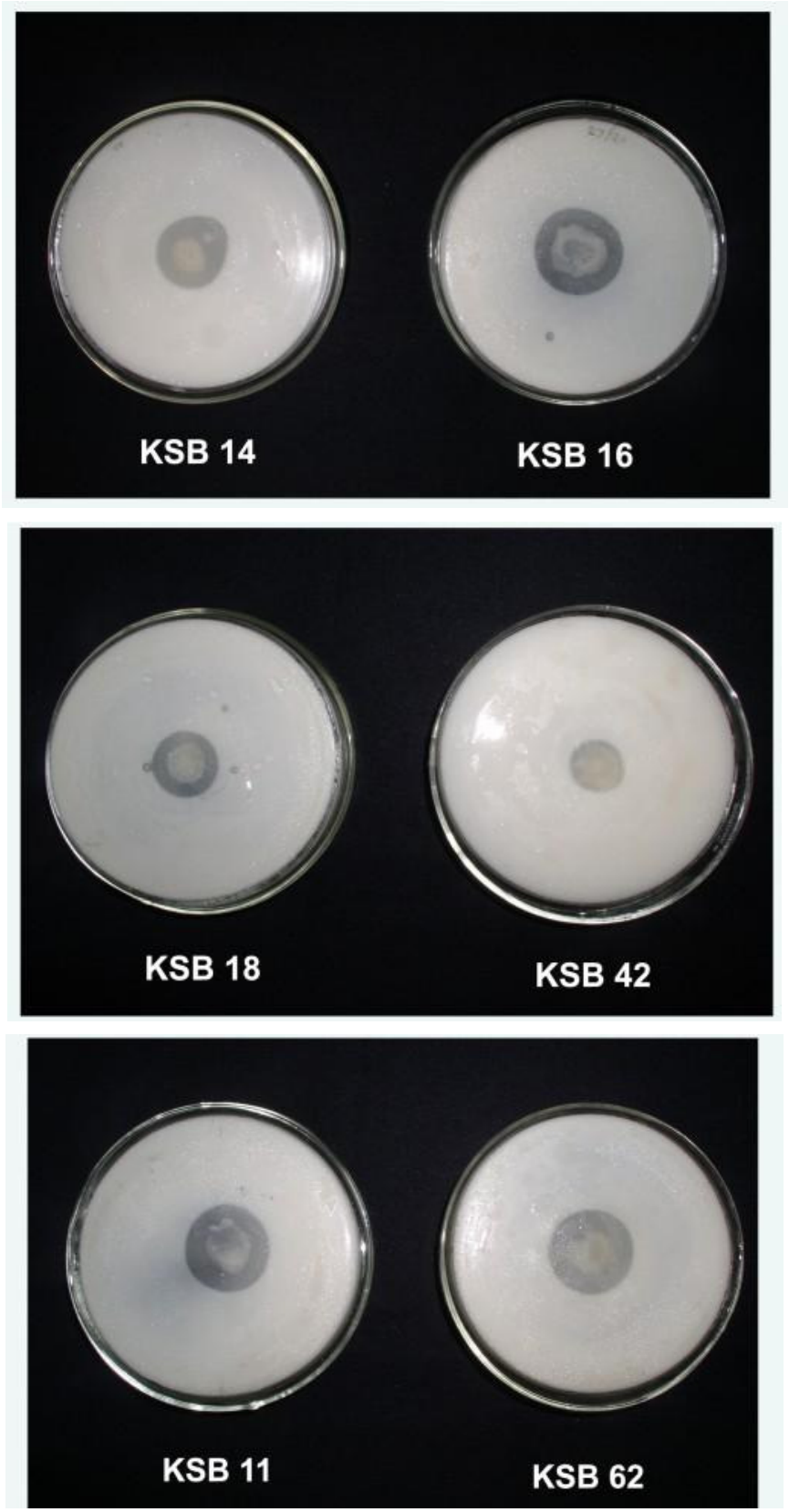


Plate 1. Potassium solubilizing bacteria showing solubilization zone on Aleksandrov medium

4.5 ORGANIC ACID PRODUCTION BY THE ISOLATES

The organic acids profile of all the isolates of potassium solubilizers was analyzed by descending chromatography and results are presented in Table 4.5. All the strains found to produce one or the other organic acid tested. It was found that the citric acid and oxalic acid are found to be the most common organic acid produced by all the 30 isolates. The other organic acid produced by the strains include malic acid (seven strains), succinic acid (five strains) and tartaric acid (three strains).

4.6 POLYSACCHARIDE PRODUCTION

The polysaccharide production by potassium solubilizing isolates was visually assessed on glucose minimal agar media and were categorized into four groups as high, moderate, low and no polysaccharide producers (Table 4.7). Three isolates KSB11, KSB42, KSB62 exhibited higher amount of polysaccharide production, six isolates produce moderate polysaccharide and remaining isolates produce low polysaccharide.

4.7 QUANTITATIVE ESTIMATION OF Pi RELEASED BY THE KSB ISOLATES

The extent of Pi released by the selected isolates in the broth were presented here. The results of the Table 4.6 revealed that the release Pi was ranging from 5.72 to 12.27 per cent and 7 isolates produced only 10 per cent Pi in the broth significantly increased release of Pi was noticed in KSB 33 (12.27%) closely followed by KSB 35 (11.87%), KSB 18 (11.60%) and KSB 42 (11.20%) over other isolates.

4.8 PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCE BY THE ISOLATES

All the isolates were examined for the productions IAA and GA on Luria agar supplemented with SDS (0.06%) and glycerol (1%). Based on the development of red colour on the filter paper or green fluorescence under UV light, 30 isolates were considered as positive for IAA and GA production (Table 4.7). All the isolates were earlier identified as K solubilizers. These isolates were further subjected to quantitative determination of the IAA and GA.

4.9 QUANTITATIVE DETERMINATION OF IAA AND GA PRODUCED BY THE ISOLATES

The amount of IAA and GA produced by the 30 isolates was determined at 10 DAI and the results are presented in Table 4.8. The amount of IAA produced by different strains ranged from 1.10 to 16.50 µg/25 ml broth. Among the isolates examined KSB14 was found to produce the high amount of IAA 16.50 µg/25 ml broth followed by KSB42 12.43 µg/25 ml broth. Both of which were significantly superior over all other strains but were on par among themselves, while five isolates should more than 10 µg of IAA/25 ml broth four produced in the range 10-7 µg and the remaining twenty-one showed less than 5 µg of IAA. The amount of GA produced by the strain ranged from 0.60 to 3.29 µg/25 ml broth (Table 4.8). Among the isolates KSB14 produced the highest amount of GA 3.29 (µg/25 ml) which was however on par with KSB 18, KSB 42 and KSB16 (3.27 µg/25 ml, 3.02 µg/25 ml and 2.62 µg/25 ml respectively but was significantly superior over rest the of isolates, while 18 isolates produced less than 1 µg/25 ml broth showed between 1.67 to 2.00 µg/25 ml and three showed more than 3.02 µg/25 ml of GA in broth.

4.10 *IN VITRO* BIOCONTROL POTENTIAL OF THE ISOLATES

All the isolates were screened for their ability to inhibit the growth of plant pathogenic fungus *Macrophomina phaseoli* on potato dextrose agar (PDA) medium by the dual culture technique while 5 isolates showed inhibition of the pathogen the remaining isolates did not show any inhibition. The results obtained are presented in Table 4.9. Out of the 30 isolates, 5 were strong inhibitors 2 were moderate and remaining were weak inhibitors of *Macrophomina phaseoli*.

Table 4.3 Details of zone of solubilization by KSB isolates

Sl. No.	Strain	Zone of solubilization (cm)
1	KSB 1	0.70
2	KSB 3	0.60
3	KSB 4	0.80
4	KSB 5	0.70
5	KSB 7	0.90
6	KSB 10	0.69
7	KSB 11	1.30
8	KSB 14	1.00
9	KSB 15	0.72
10	KSB 16	1.20
11	KSB 18	1.00
12	KSB 21	0.80
13	KSB 22	0.91
14	KSB 23	0.82
15	KSB 25	0.68
16	KSB 27	0.60
17	KSB 29	0.68
18	KSB 32	0.90
19	KSB 33	1.00
20	KSB 35	1.00
21	KSB 42	1.30
22	KSB 45	0.90
23	KSB 47	1.00
24	KSB 48	0.70
25	KSB 50	0.70
26	KSB 55	0.80
27	KSB 62	1.30
28	KSB 64	0.60
29	KSB 67	0.70
30	KSB 69	0.70

Table 4.4 Solubilization of muscovite mica by the potassium solubilizing bacterial (KSB) isolates on release of K

Sl. No.	Isolate	7 DAI (µg/ml)	15 DAI (µg/ml)	20 DAI (µg/ml)
1	Control	0.06	0.07	0.07
2	KSB 1	3.42	5.55	5.89
3	KSB 3	2.67	7.93	7.91
4	KSB 4	3.25	7.63	7.73
5	KSB 5	3.58	6.50	6.90
6	KSB 7	2.66	7.72	8.12
7	KSB 10	2.85	7.90	7.99
8	KSB 11	25.09	42.89	44.49
9	KSB 14	6.66	18.79	29.93
10	KSB 15	3.11	8.40	8.72
11	KSB 16	7.40	28.49	31.91
12	KSB 18	6.66	19.19	23.03
13	KSB 21	2.85	7.90	7.99
14	KSB 22	3.11	9.40	10.72
15	KSB 23	2.65	7.72	10.00
16	KSB 25	0.92	1.20	2.41
17	KSB 27	2.70	6.50	6.39
18	KSB 29	0.91	2.76	2.86
19	KSB 32	2.85	7.90	7.50
20	KSB 33	25.63	30.77	31.90
21	KSB 35	6.66	15.54	20.50
22	KSB 42	7.31	34.56	37.07
23	KSB 45	1.19	4.59	6.39
24	KSB 47	5.40	10.19	22.50
25	KSB 48	0.58	2.51	5.41
26	KSB 50	1.06	3.64	4.51
27	KSB 55	2.50	7.95	7.90
28	KSB 62	9.89	25.34	36.59
29	KSB 64	1.19	2.47	5.85
30	KSB 67	0.78	1.55	3.87
31	KSB 69	0.66	1.56	4.78
	S.Em±	0.16	0.21	0.18
	CD @ 1%	0.58	0.77	0.67

DAI – Days after inoculation

Table 4.5 The profile of organic acid production by the potassium solubilizing bacteria

Sl. No.	Strain	Oxalic acid	Malic acid	Citric acid	Succinic acid	Tarteric acid
1	KSB 1	+	-	+	-	-
2	KSB 3	+	+	+	-	-
3	KSB 4	+	-	+	-	-
4	KSB 5	+	-	+	+	-
5	KSB 7	+	-	+	-	-
6	KSB 10	+	-	+	-	+
7	KSB 11	+	-	+	-	+
8	KSB 14	+	-	+	-	-
9	KSB 15	+	-	+	-	-
10	KSB 16	+	+	+	+	-
11	KSB 18	+	-	+	-	-
12	KSB 21	+	-	+	-	-
13	KSB 22	+	-	+	+	-
14	KSB 23	+	-	+	+	+
15	KSB 25	+	+	+	-	-
16	KSB 27	+	-	+	-	-
17	KSB 29	+	-	+	-	-
18	KSB 32	+	-	+	-	-
19	KSB 33	+	+	+	-	-
20	KSB 35	+	-	+	-	-
21	KSB 42	+	+	+	-	-
22	KSB 45	+	-	+	+	-
23	KSB 47	+	+	+	-	-
24	KSB 48	+	-	+	-	-
25	KSB 50	+	-	+	-	-
26	KSB 55	+	-	+	-	-
27	KSB 62	+	+	+	-	-
28	KSB 64	+	-	+	-	-
29	KSB 67	+	-	+	-	-
30	KSB 69	+	-	+	-	-

Table 4.6 Per cent Pi released by potassium solubilizing bacterial isolates

Sl. No.	Isolate	% Pi released		
		7 DAI	15 DAI	20 DAI
1	KSB 1	0.98	2.61	7.38
2	KSB 3	1.13	2.40	6.71
3	KSB 4	0.97	2.99	5.98
4	KSB 5	1.18	3.53	7.05
5	KSB 7	1.25	3.68	8.91
6	KSB 10	1.81	2.84	5.72
7	KSB 11	3.66	6.53	10.00
8	KSB 14	3.19	6.39	9.80
9	KSB 15	1.74	2.95	8.92
10	KSB 16	2.39	5.53	10.00
11	KSB 18	1.99	6.79	11.60
12	KSB 21	0.99	2.71	7.48
13	KSB 22	1.19	3.54	7.06
14	KSB 23	1.28	3.10	6.22
15	KSB 25	1.55	2.06	6.16
16	KSB 27	1.18	2.77	6.11
17	KSB 29	2.68	4.78	8.13
18	KSB 32	1.27	2.96	8.62
19	KSB 33	3.90	6.46	12.27
20	KSB 35	3.86	7.33	11.87
21	KSB 42	3.33	6.99	11.20
22	KSB 45	2.39	6.06	8.80
23	KSB 47	2.79	5.86	10.13
24	KSB 48	2.79	6.13	8.87
25	KSB 50	1.74	2.99	7.54
26	KSB 55	1.18	3.53	8.00
27	KSB 62	2.79	5.46	9.87
28	KSB 64	1.12	2.50	7.00
29	KSB 67	0.99	2.71	7.38
30	KSB 69	1.25	3.68	8.12
	S.Em±	1.13	0.04	0.08
	CD @ 1%	3.42	0.14	0.30

DAI – Days after inoculation

All the values obtained after deducting the Pi present in the respective uninoculated controls

Table 4.7 Production of growth promoting substances and polysaccharide by potassium solubilizing bacteria (KSB)

Sl. No.	Isolate	IAA	GA	Polysaccharide
1	KSB 1	+	+	+
2	KSB 3	+	+	+
3	KSB 4	+	+	+
4	KSB 5	+	+	+
5	KSB 7	+	+	+
6	KSB 10	+	+	+
7	KSB 11	+	+	+++
8	KSB 14	+	+	++
9	KSB 15	+	+	+
10	KSB 16	+	+	++
11	KSB 18	+	+	++
12	KSB 21	+	+	+
13	KSB 22	+	+	+
14	KSB 23	+	+	+
15	KSB 25	+	+	+
16	KSB 27	+	+	+
17	KSB 29	+	+	+
18	KSB 32	+	+	+
19	KSB 33	+	+	++
20	KSB 35	+	+	++
21	KSB 42	+	+	+++
22	KSB 45	+	+	+
23	KSB 47	+	+	++
24	KSB 48	+	+	+
25	KSB 50	+	+	+
26	KSB 55	+	+	+
27	KSB 62	+	+	+++
28	KSB 64	+	+	+
29	KSB 67	+	+	+
30	KSB 69	+	+	+

IAA : Indole acetic acid
GA : Gibberllic acid

+++ : High polysaccharide
++ : Moderate polysaccharide
+ : Low polysaccharide
- : No polysaccharide

Table 4.8 Production of growth promoting substance by potassium solubilizing bacteria (KSB)

Sl. No.	Isolate	IAA (µg/25 ml)	GA (µg/25 ml)
1	KSB 1	1.50	0.60
2	KSB 3	1.10	0.73
3	KSB 4	3.30	0.07
4	KSB 5	5.31	0.09
5	KSB 7	1.27	0.29
6	KSB 10	3.67	0.39
7	KSB 11	8.57	1.72
8	KSB 14	16.50	3.29
9	KSB 15	4.52	1.67
10	KSB 16	9.53	2.62
11	KSB 18	13.83	3.27
12	KSB 21	4.63	0.81
13	KSB 22	4.00	1.23
14	KSB 23	2.23	0.61
15	KSB 25	5.14	0.63
16	KSB 27	3.30	0.07
17	KSB 29	4.67	1.67
18	KSB 32	7.97	0.85
19	KSB 33	11.50	2.00
20	KSB 35	10.63	2.57
21	KSB 42	12.43	3.02
22	KSB 45	5.37	0.63
23	KSB 47	11.70	2.33
24	KSB 48	6.33	0.70
25	KSB 50	1.10	0.73
26	KSB 55	1.40	0.60
27	KSB 62	8.80	1.93
28	KSB 64	1.60	0.67
29	KSB 67	3.80	0.60
30	KSB 69	5.53	0.95
	S.Em±	0.17	0.02
	CD @ 1%	0.65	0.07

Table 4.9 Biocontrol potential of the potassium solubilizing bacteria (KSB) against plant pathogenic fungi

Sl. No.	Isolates	Fungal pathogen *Macrophomina phaseoli*
1	KSB 1	+
2	KSB 3	-
3	KSB 4	-
4	KSB 5	-
5	KSB 7	-
6	KSB 10	+
7	KSB 11	-
8	KSB 14	-
9	KSB 15	-
10	KSB 16	-
11	KSB 18	+
12	KSB 21	-
13	KSB 22	-
14	KSB 23	-
15	KSB 25	-
16	KSB 27	-
17	KSB 29	-
18	KSB 32	-
19	KSB 33	-
20	KSB 35	-
21	KSB 42	-
22	KSB 45	+
23	KSB 47	-
24	KSB 48	-
25	KSB 50	-
26	KSB 55	-
27	KSB 62	-
28	KSB 64	-
29	KSB 67	-
30	KSB 69	+

4.11 EFFECT OF INOCULATION OF SELECTED POTASSIUM SOLUBILIZING BACTERIA ON GROWTH YIELD AND K UPTAKE OF MAIZE PLANTS

To study the effect of inoculation of 9 efficient isolates of K solubilizing bacteria on growth, yield and K uptake of maize plants, a pot culture experiment was conducted and the results recorded at 55 DAS and at harvest are presented in Table 4.10 to 4.15.

4.12 ROOT LENGTH

Significant differences in the root length of maize plants was observed at 55 days of plant growth due to various inoculation treatments and fertilizer application (Table 4.10).

The treatment receiving inoculation of *Bacillus* isolate KSB11 recorded maximum root length (59.66 cm) and was significantly superior over all other inoculation treatments and 25 per cent RDK (50.67 cm) but was on par with 75 and 100 per cent RDK treatments (59.67 and 67.33 cm) respectively. However, all the other inoculated treatments showed significant increase in root growth over absolute control. Six of the isolates including reference strain recorded significant increase in root length over 25 per cent RDK.

4.13 SHOOT LENGTH

All the treatment receiving inoculation of bacteria increased the shoot length of maize plants significantly over absolute control, but on par with uninoculated fertilizer control.

Among the inoculated treatments the reference strain showed maximum shoot length of maize at 55 days of growth (67.15 cm) which were significantly superior over the all other inoculated treatments and the absolute control. However, it was on par with rest of the treatments including the three K levels.

The highest shoot length was observed with treatment receiving 100 per cent recommended dose of K (69.22 cm) which was however on par with 25 and 75 RDK and three isolates KSB11, KSB62 and KSB42 (65.17, 65.28 and 64.24 cm respectively).

4.14 STEM GIRTH

Two isolates (KSB62 and KSB11) showed significantly higher stem girth 6.00 and 6.28 cm respectively over the absolute control (Table 4.10). However, these treatments were on par with each other an with treatments receiving 75 per cent RDK 6.17 cm.

4.15 NUMBER OF LEAVES PER PLANT

Among the inoculation treatments highest number of leaves was recorded with treatment receiving strain KSB11 (8.67 leaves/plant) followed by KSB62 (8.50 leaves/plant) and KSB42 (8.00 leaves/plant) which were on par among themselves and also with all the three levels of K. No significant differences existed between the levels of K with respect to number of leaves per plant. However, highest number of leaves among K levels was observed with treatment receiving 100 per cent RDK 9.00 leaves/plant).

4.16 CHLOROPHYLL CONTENT

The data on chlorophyll content of maize plants at 55 DAS as influenced by KSB are presented in Table 4.10. All the inoculated bacteria enhanced the chlorophyll content significantly over absolute control. Among the inoculated treatment KSB 11 recorded maximum chlorophyll content of 2.00 mg/g of tissue followed by reference strain and KSB62 (1.95 and 1.90 mg/g of tissue respectively). While all the inoculation treatments recorded significantly superior chlorophyll content over 25 per cent RDK control. However, highest chlorophyll content among K levels was observed with treatment receiving 100 per cent RDK 2.58 mg/g of tissue.

4.17 DRY MATTER PRODUCTION

The data on shoot root and total dry matter production by maize plants at 55 days of plant growth are presented in Table 4.11.

Plate 2. General view of pot culture experiment

Plate 2. General view of pot culture experiment

Table 4.10 Effect of efficient potassium solubilizing bacteria (KSB) on growth of maize plant at 55 DAS

Tr. No.	Treatments	Root length (cm)	Shoot length (cm)	No. of leaves/ plant	Stem girth (cm)	Chlorophyll content (mg/g) of tissue
T_1	Absolute control (no inoculation, no fertilizer K)	40.33	59.50	7.83	5.37	0.79
T_2	Potassium control 1 (25% RDK)	50.67	63.58	8.33	5.57	1.27
T_3	Potassium control 2 (75% RDK)	59.67	66.95	8.50	6.17	1.96
T_4	Potassium control 3 (100% RDK)	67.33	69.22	9.00	6.62	2.58
T_5	*Frateuria aurantia* (Reference strain)	53.67	67.15	8.00	6.15	1.95
T_6	Isolate 11	59.66	65.17	8.67	6.28	2.00
T_7	Isolate 14	50.33	62.10	7.33	5.50	1.27
T_8	Isolate 16	50.00	60.47	7.50	5.65	1.29
T_9	Isolate 18	50.33	63.18	7.67	5.60	1.62
T_{10}	Isolate 33	50.33	63.83	7.83	5.58	1.81
T_{11}	Isolate 35	51.33	61.98	7.83	5.62	1.70
T_{12}	Isolate 42	54.33	64.24	8.00	5.07	1.98
T_{13}	Isolate 47	53.00	60.00	7.50	5.85	1.73
T_{14}	Isolate 62	55.00	65.28	8.50	6.00	1.90
	S.Em±	0.37	0.42	0.56	0.11	0.08
	CD @ 1%	1.44	1.63	2.20	0.44	0.01

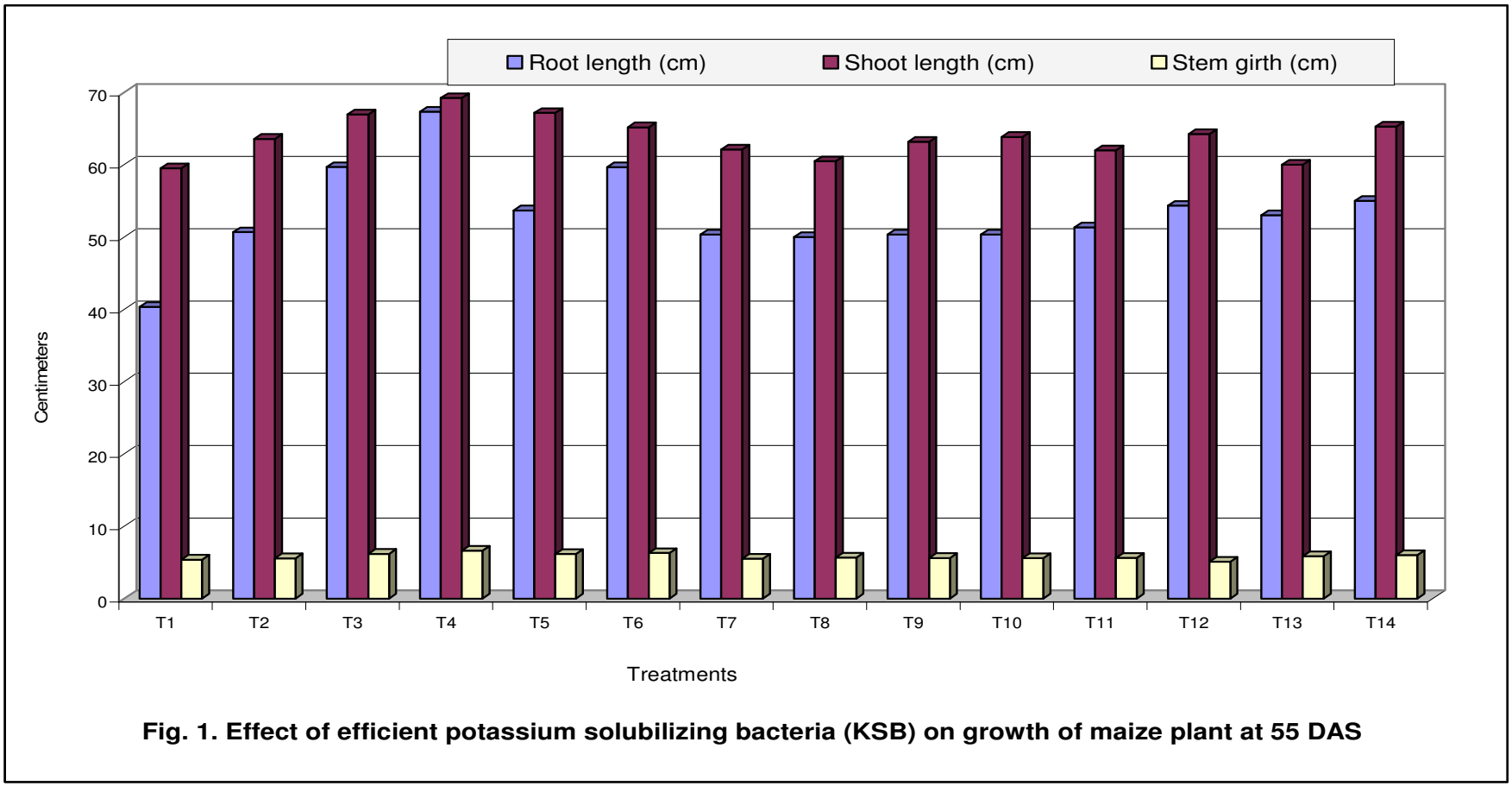


Fig. 1. Effect of efficient potassium solubilizing bacteria (KSB) on growth of maize plant at 55 DAS

Fig. 1. Effect of efficient potassium solubilizing bacteria (KSB) on growth of maize plant at 55 DAS

4.18 SHOOT DRY MATTER

All the inoculated treatments recorded significantly higher shoot dry matter over the absolute control. Treatment receiving inoculation with isolates KSB 11 followed by the reference strain recorded maximum shoot dry matter (24.73 g and 23.20 g) respectively and both were on par with treatments receiving 75 per cent of recommended dose of K (25.68 g). The treatments receiving 75 and 100 per cent K were on par with each other but both were significantly superior overall other treatments (Table 4.11).

4.19 ROOT DRY MATTER

At 55 days of plant growth, all the inoculation treatments, showed significant increase in root dry matter over absolute control (Table 4.11). All the K levels also showed significantly higher root dry matter than control. Among the inoculation treatments maximum root dry matter was recorded with KSB 11 (3.58 g) which was significantly superior over all other inoculation treatments. All other inoculation treatments including reference strain was also on par with 25 per cent RDK (2.43 g). The highest root dry matter among all the treatments was recorded with 100 per cent RDK (4.17 g/plant) followed by 75 per cent RDK (4.04 g/plant) both of which were significantly superior overall other treatments but were on par among themselves.

4.20 TOTAL DRY MATTER

Among the inoculation treatments isolate KSB11 recorded maximum total dry matter (28.31 g) at 55 days of plant growth which was significantly superior overall other inoculation treatments except reference strain (25.43 g) with which it was on par with while KSB 33 was on par with 25 per cent RDK (18.11 g). Isolate KSB 11 was on par with both 25 and 75 per cent RDK (28.72 g). No significant differences existed between 25 and 75 per cent RDK levels and 100 per cent RDK levels (Table 4.12). However 75 and 100 per cent RDK levels recorded maximum total dry matter which were significantly superior over all other treatments.

4.21 POPULATION OF KSB IN THE RHIZOSPHERE AND AVAILABLE K CONTENT OF SOIL AT 55 DAS

The data on the population of KSB in the rhizosphere of maize and the available K content in soil at 55 DAS as influenced by inoculation with KSB strains and fertilizer control are presented in Table 41.2

The application of potash fertilizer (without inoculation) did not influence the population of KSB in the rhizosphere of maize. However, the inoculation of KSB strains increased the population of KSB in the rhizosphere by (38.67 cfu x 10^4/g soil). All the KSB strains recorded significantly higher population of KSB in rhizosphere over absolute control and 3 levels of K control. The highest KSB population was recorded in the treatment receiving strain KSB 62 which was significantly superior over all other strains. The available K content in soil at 55 DAS was significantly higher in treatment receiving KSB inoculation and fertilizer control as compared to absolute control (Table 4.12), while 100 per cent K control (206.00 kg/ha) was significantly superior over absolute control (138.67 kg/ha). All the KSB strains showed significantly higher available K in soil over absolute control, where as three strains *viz.,* KSB 62, KSB 11, KSB 42 showed significantly higher available K in soil than 25 per cent K control. Among the stains of KSB 62 (390.33 kg/ha) recorded the highest available K content in soil followed by KSB 11, KSB 18 and KSB 42.

4.22 DRY MATTER CONTENT AT HARVEST

The shoot, root and total dry matter content of maize plants at harvest as influenced by various inoculation and fertilizer treatments are given in Table 4.13.

All the inoculation treatments showed significant increase in shoot dry matter over absolute control. Among the inoculation treatment isolate KSB 11 and KSB 62 (102.53 and 101.60 g respectively) recorded maximum shoot dry weight and were significantly superior over all other inoculation treatments. However, they were on par among themselves and also with 25 per cent of RDK (103.10 g). The other K levels *viz.,*75 and 100 per cent RDK were significantly superior over all the inoculation treatments.

L E G E N D

Treatment No.	Treatments
T_1	Absolute control (No inoculation, no fertilizer K)
T_2	Potassium control 1 (25% RDK)
T_3	Potassium control 2 (75% RDK)
T_4	Potassium control 3 (100% RDK)
T_5	*Frateuria aurantia* (Reference strain)
T_6	Isolate KSB 11
T_{12}	Isolate KSB 42
T_{13}	Isolate KSB 47
T_{14}	Isolate KSB 62

Plate 3. Effect of KSB on plant growth at 55 DAS

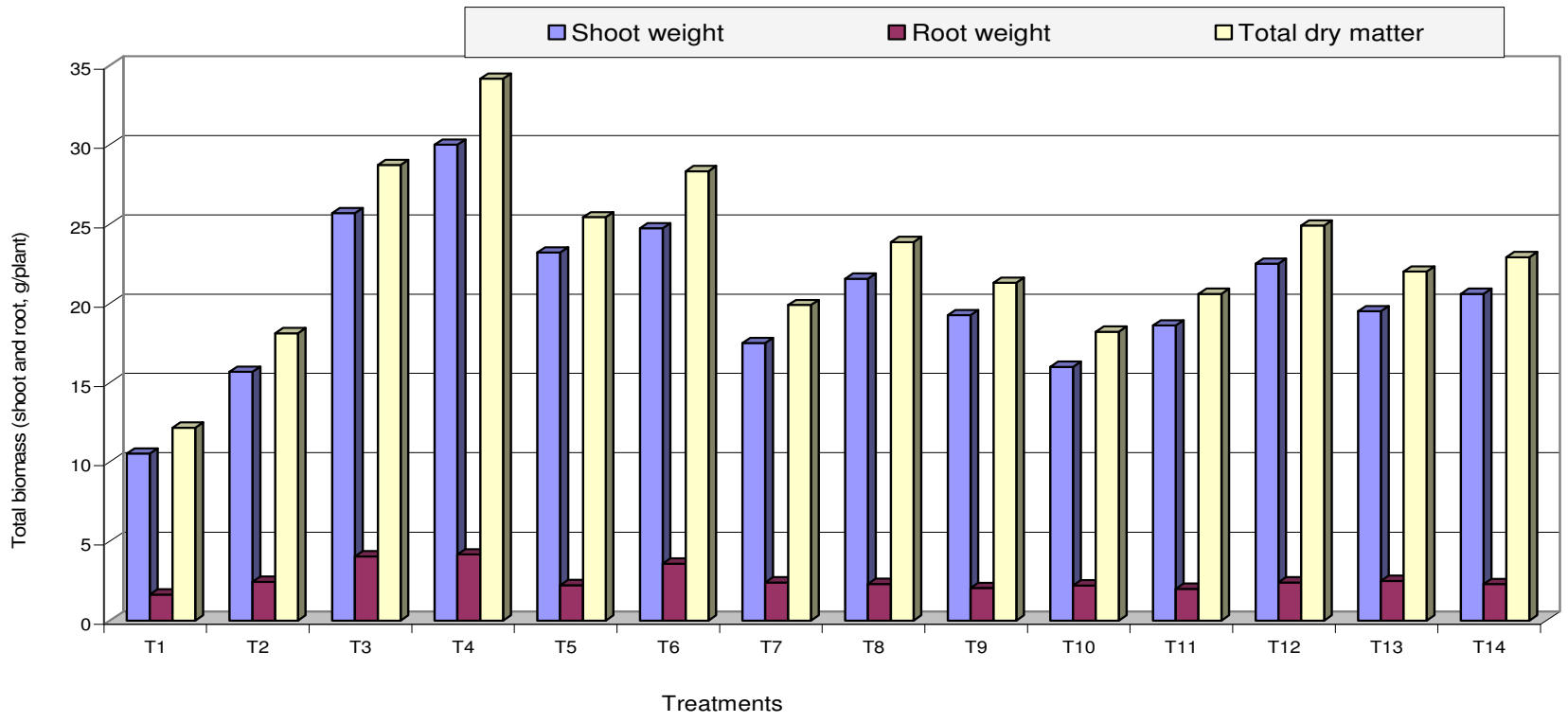


Fig. 2. Effect of efficient potassium solubilizing bacteria (KSB) dry matter content of plant at 55 DAS (g/plant)

Fig. 2. Effect of efficient potassium solubilizing bacteria (KSB) dry matter content of plant at 55 DAS (g/plant)

Table 4.11 Effect of efficient potassium solubilizing bacteria (KSB) dry matter content of plant at 55 DAS (g/plant)

Tr. No.	Treatments	Shoot weight	Root weight	Total dry matter
T_1	Absolute control (no inoculation, no fertilizer K)	10.52	1.64	12.16
T_2	Potassium control 1 (25% RDK)	15.68	2.43	18.11
T_3	Potassium control 2 (75% RDK)	25.68	4.04	28.72
T_4	Potassium control 3 (100% RDK)	30.00	4.17	34.17
T_5	*Frateuria aurantia* (Reference strain)	23.20	2.23	25.43
T_6	Isolate 11	24.73	3.58	28.33
T_7	Isolate 14	17.49	2.40	19.89
T_8	Isolate 16	21.55	2.31	23.86
T_9	Isolate 18	19.25	2.05	21.30
T_{10}	Isolate 33	16.00	2.21	18.21
T_{11}	Isolate 35	18.60	2.00	20.60
T_{12}	Isolate 42	22.50	2.40	24.90
T_{13}	Isolate 47	19.50	2.50	22.00
T_{14}	Isolate 62	20.60	2.30	22.90
	S.Em±	0.48	0.07	0.30
	CD @ 1%	1.87	0.28	1.18

Table 4.12 Population of potassium solubilizing bacteria (KSB) in the rhizosphere of maize and available K content in soil at 55 DAS as influenced by KSB strains

Tr. No.	Treatments	KSB population (cfu x 10^4/g soil)	Available K in soil (kg/ha)
T_1	Absolute control (no inoculation, no fertilizer K)	10.57	138.67
T_2	Potassium control 1 (25% RDK)	12.33	143.67
T_3	Potassium control 2 (75% RDK)	14.33	180.33
T_4	Potassium control 3 (100% RDK)	26.67	206.00
T_5	*Frateuria aurantia* (Reference strain)	29.33	141.67
T_6	Isolate 11	30.67	250.33
T_7	Isolate 14	29.33	214.67
T_8	Isolate 16	17.67	229.67
T_9	Isolate 18	35.67	243.67
T_{10}	Isolate 33	30.00	231.00
T_{11}	Isolate 35	25.33	170.33
T_{12}	Isolate 42	29.33	232.00
T_{13}	Isolate 47	25.67	215.00
T_{14}	Isolate 62	38.67	390.33
	S.Em±	0.31	0.66
	CD @ 1%	0.21	2.58

Table 4.13 Effect of efficient (KSB) potassium solubilizing bacteria on dry matter content of maize plants at harvest

Tr. No.	Treatments	Shoot weight (g/plant)	Root weight (g/plant)	Total dry matter (g/plant)
T_1	Absolute control (no inoculation, no fertilizer K)	87.82	13.40	101.23
T_2	Potassium control 1 (25% RDK)	103.10	18.90	122.00
T_3	Potassium control 2 (75% RDK)	105.27	22.00	127.20
T_4	Potassium control 3 (100% RDK)	107.97	23.00	130.93
T_5	*Frateuria aurantia* (Reference strain)	97.30	16.10	113.47
T_6	Isolate 11	102.53	16.73	119.37
T_7	Isolate 14	93.33	14.67	107.97
T_8	Isolate 16	92.80	14.50	107.33
T_9	Isolate 18	90.53	13.00	103.73
T_{10}	Isolate 33	91.33	12.93	104.33
T_{11}	Isolate 35	89.17	13.87	102.67
T_{12}	Isolate 42	98.33	16.37	114.33
T_{13}	Isolate 47	89.17	12.60	102.33
T_{14}	Isolate 62	101.60	15.50	117.10
	S.Em±	0.27	0.21	0.34
	CD @ 1%	1.05	0.80	1.32

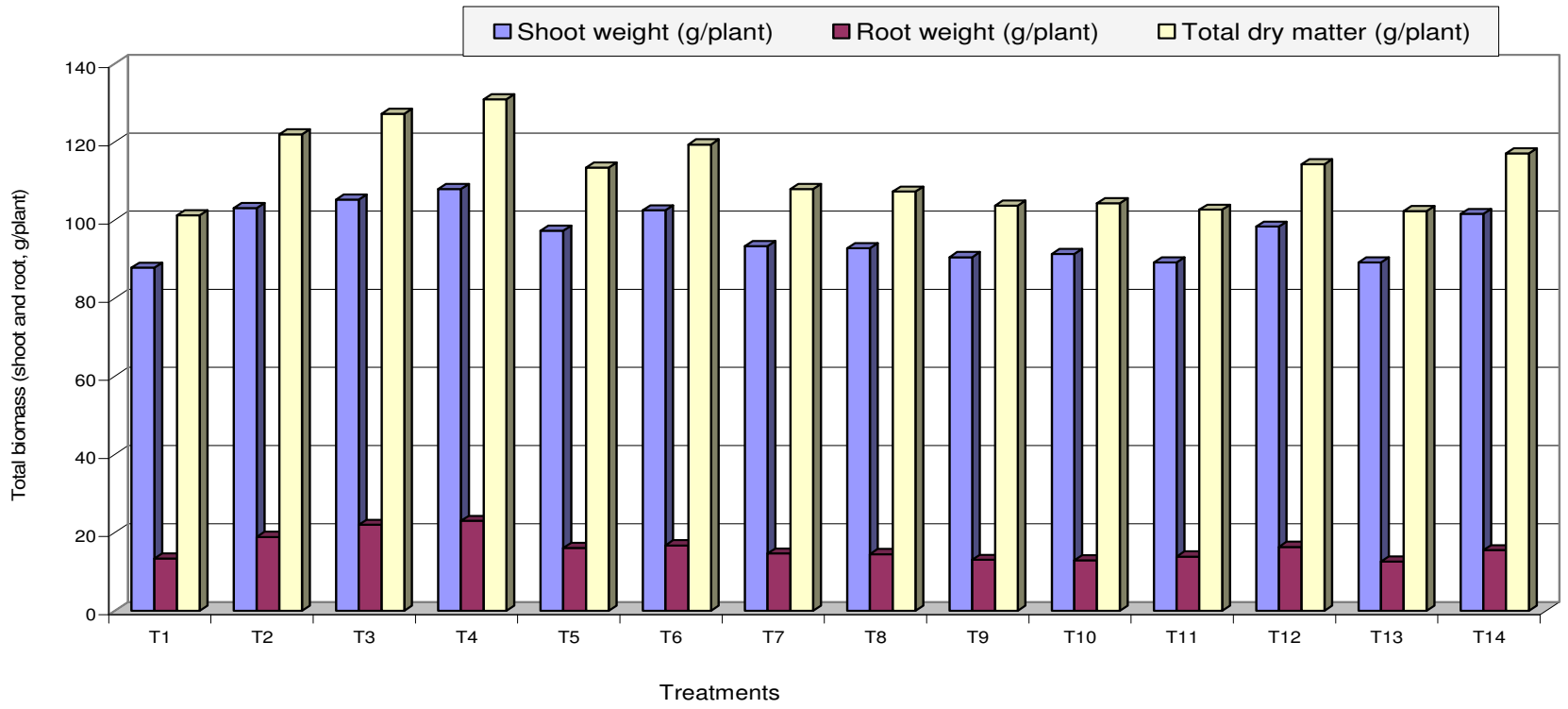


Fig. 3. Effect of efficient (KSB) potassium solubilizing bacteria on dry matter content of maize plants at harvest

Fig. 3. Effect of efficient (KSB) potassium solubilizing bacteria on dry matter content of maize plants at harvest

Significant differences existed between the treatments with respect to root dry matter of maize plants at harvest only four out of the 10 inoculation treatments recorded significant increase in root dry weight over absolute control, whereas all other were on par with it. Among the inoculation treatments isolate KSB 11 recorded maximum root dry weight (16.73 g) and was significantly superior over all other inoculation treatment except KSB 62 (15.50 g) and reference strain (16.10 g) with which it was on par. However the root dry weight recorded in all the three K levels was significantly higher over that recorded with inoculation treatments.

The total dry matter content of maize plants at harvest was significantly higher in all the inoculation treatments over absolute control (Table 4.13).

Isolate KSB 11 among the inoculation treatments recorded maximum total dry matter (119.37 g) closely followed by KSB 62 (117.1 g) both of which were on par with each other but were significantly superior over all other inoculation treatments. All the three K level recorded significantly higher total dry matter over all the inoculation treatments.

4.23 YIELD AND YIELD PARAMETERS

The data on yield and yield parameters of maize plants as influenced by inoculated treatments and fertilizer K are presented in Table 4.14.

4.24 COB WEIGHT

Five out of ten inoculation treatments showed significant increase in cob weight over absolute control where as the remaining isolates were either on par with it or significantly lower (Table 4.14).

Among the inoculation treatments KSB 11 recorded maximum cob weight (140.67 g) closely followed by KSB 42 (139.67 g) and KSB 62 (138.33 g) all of which were on par among themselves and with 25 per cent RDK. Treatments receiving KSB11, KSB 62 and KSB 42 and reference strain showed cob weight that was on par with 25 per cent RDK, however, 75 per cent and 100 per cent RDK (148.73 g and 153.33 g respectively) recorded maximum cob weight among all the treatments.

4.25 GRAIN YIELD

The grain yield of maize plants was increased significantly by six out of 10 strains inoculated as compared to absolute control (Table 4.14). Isolate KSB 11 among the inoculation treatments recorded maximum grain yield per pot (51.33 g) which was however on par with KSB 42 and reference strain (49.67 g, 49.67 g) respectively and all of them were on par with 25 per cent RDK (49.43 g) K at 75 and 100 per cent recommended levels which recorded significantly higher grain yield of 63.33 g and 63.77 g over all other treatments. No significant difference existed between 75 per cent and 100 per cent RDK.

4.26 HUNDRED GRAIN WEIGHT

Hundred grain weight was found to be increased significantly due to inoculation of six out of 10 strains and application of fertilizer K at all level (Table 4.14). Among the inoculation treatments KSB 11 recorded maximum seed weight 23.73 g, which was significantly superior overall inoculation treatments except KSB 16, KSB 42 and KSB 62 and reference strain 20.07, 21.33, 21.67 and 20.57 respectively with which it was on par.

All the four strains were on par with 25 per cent RDK, but at 75 per cent and 100 per cent recommended level showed significantly higher seed weight than all other treatments.

4.27 K UPTAKE

The data on K uptake in maize plants at 55 days and at harvest of growth are presented in Table 4.15.

4.27.1 'K' uptake at 55 DAS

All the inoculation treatments, except two isolate each of KSB 16 and KSB 47 showed significantly higher 'K' uptake over absolute control.

Table 4.14 Effect of efficient (KSB) potassium solubilizing bacteria on yield and yield parameters of maize plants at harvest

Tr. No.	Treatments	Cob weight (g/plant)	Grain yield (g/plant)	100 seed weight (g)
T_1	Absolute control (no inoculation, no fertilizer K)	100.63	30.30	10.30
T_2	Potassium control 1 (25% RDK)	139.90	49.43	23.63
T_3	Potassium control 2 (75% RDK)	148.73	63.33	27.67
T_4	Potassium control 3 (100% RDK)	153.33	63.77	29.43
T_5	*Frateuria aurantia* (Reference strain)	128.33	49.67	20.57
T_6	Isolate 11	140.67	51.33	23.73
T_7	Isolate 14	95.00	25.80	9.00
T_8	Isolate 16	99.67	40.33	20.07
T_9	Isolate 18	103.47	43.00	17.00
T_{10}	Isolate 33	90.30	27.50	8.90
T_{11}	Isolate 35	98.47	29.33	8.00
T_{12}	Isolate 42	139.67	49.67	21.33
T_{13}	Isolate 47	96.33	20.33	9.83
T_{14}	Isolate 62	138.33	46.33	21.67
	S.Em±	0.33	0.29	1.28
	CD @ 1%	1.30	1.15	1.09

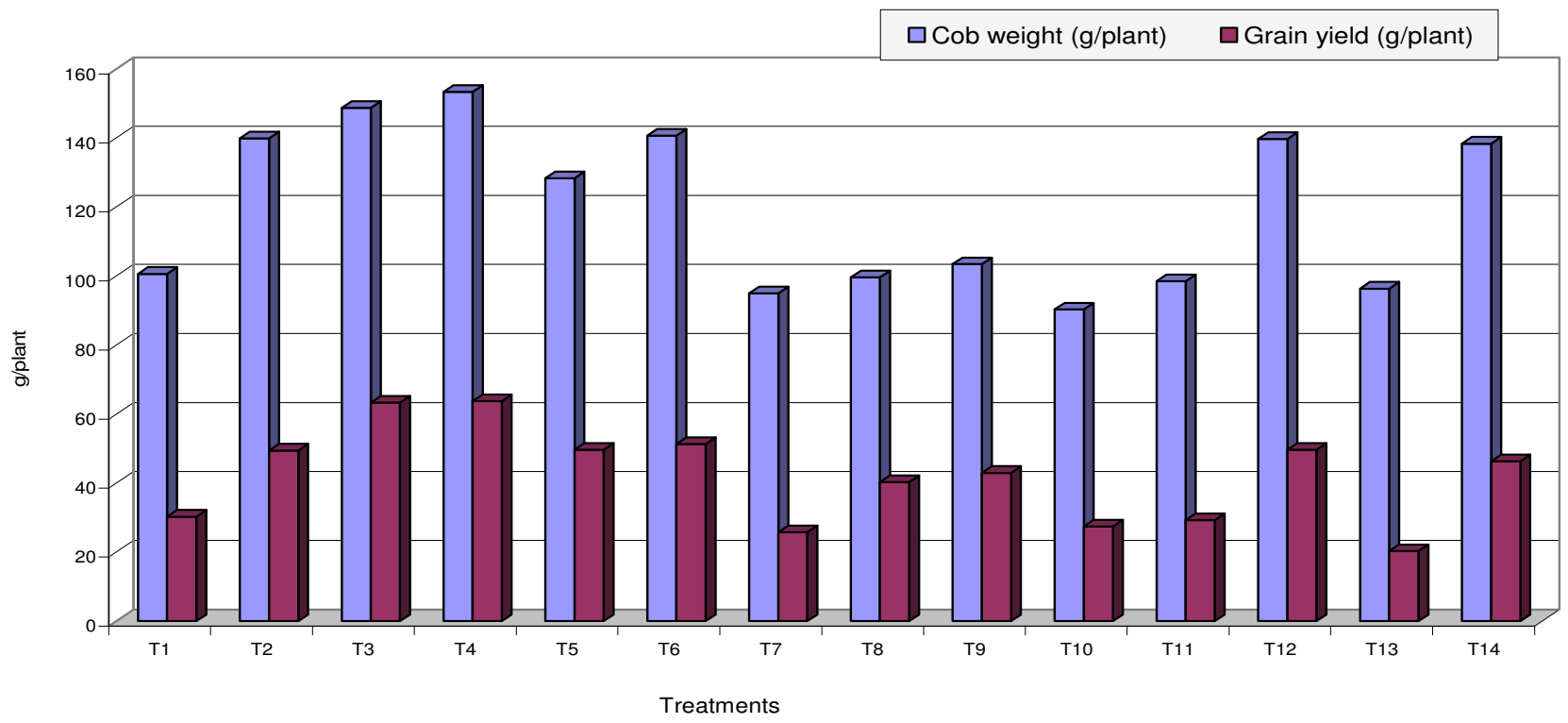
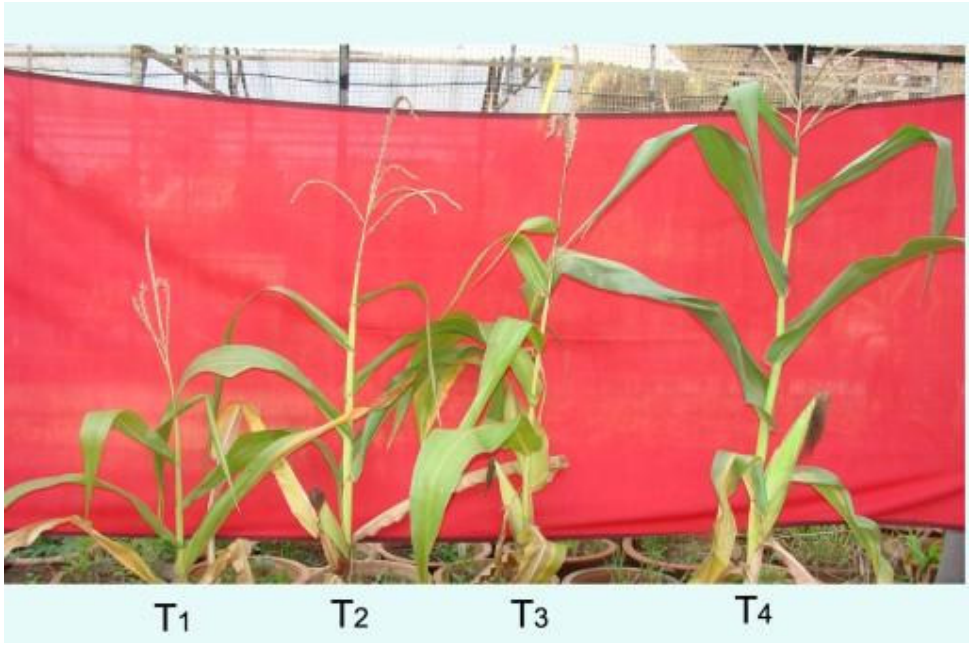


Fig. 4. Effect of efficient (KSB) potassium solubilizing bacteria on yield and yield parameters of maize plants at harvest

Fig. 4. Effect of efficient (KSB) potassium solubilizing bacteria on yield and yield parameters of maize plants at harvest

L E G E N D

Treatment No.	Treatments
T_1	Absolute control (No inoculation, no fertilizer K)
T_2	Potassium control 1 (25% RDK)
T_3	Potassium control 2 (75% RDK)
T_4	Potassium control 3 (100% RDK)
T_6	Isolate KSB 11
T_{10}	Isolate KSB 33
T_{12}	Isolate KSB 42
T_{13}	Isolate KSB 47
T_{14}	Isolate KSB 62



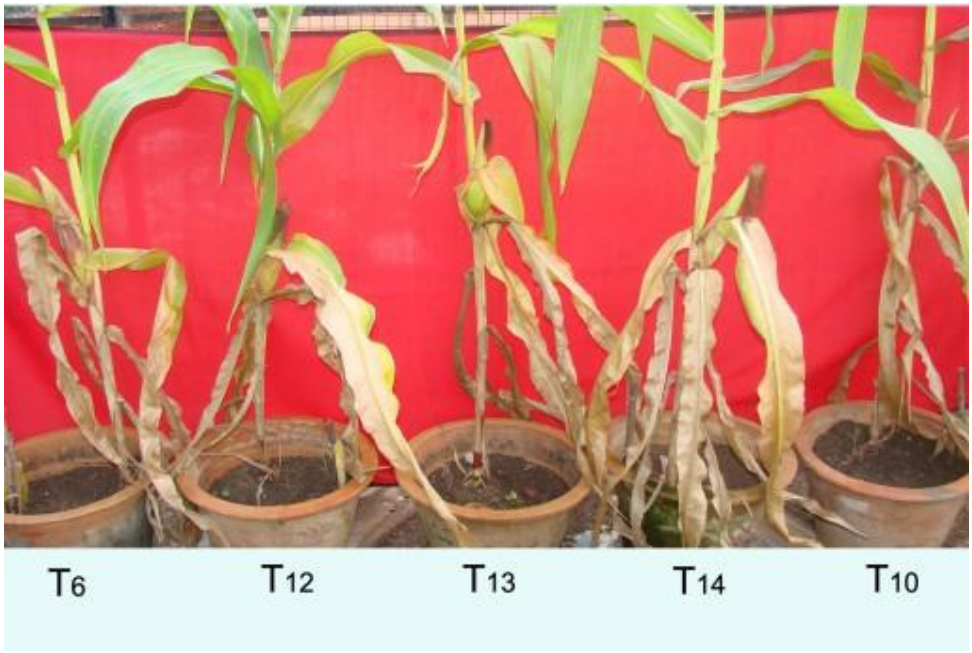


Plate 4. Effect of KSB and plant growth at harvest

Table 4.15 Effect of efficient (KSB) potassium solubilizing bacteria on K uptake of maize plants at 55 DAS and at harvest

Tr. No.	Treatments	K uptake (mg/plant)	
		55 DAS	At harvest
T_1	Absolute control (no inoculation, no fertilizer K)	166.67	215.33
T_2	Potassium control 1 (25% RDK)	257.00	370.67
T_3	Potassium control 2 (75% RDK)	259.00	390.67
T_4	Potassium control 3 (100% RDK)	300.00	534.67
T_5	*Frateuria aurantia* (Reference strain)	196.33	289.33
T_6	Isolate 11	249.33	350.33
T_7	Isolate 14	215.33	240.33
T_8	Isolate 16	166.10	238.67
T_9	Isolate 18	184.33	218.67
T_{10}	Isolate 33	200.00	220.33
T_{11}	Isolate 35	230.33	210.33
T_{12}	Isolate 42	260.33	368.67
T_{13}	Isolate 47	150.33	200.00
T_{14}	Isolate 62	253.33	290.33
	S.Em±	0.30	0.32
	CD @ 1%	1.17	1.26

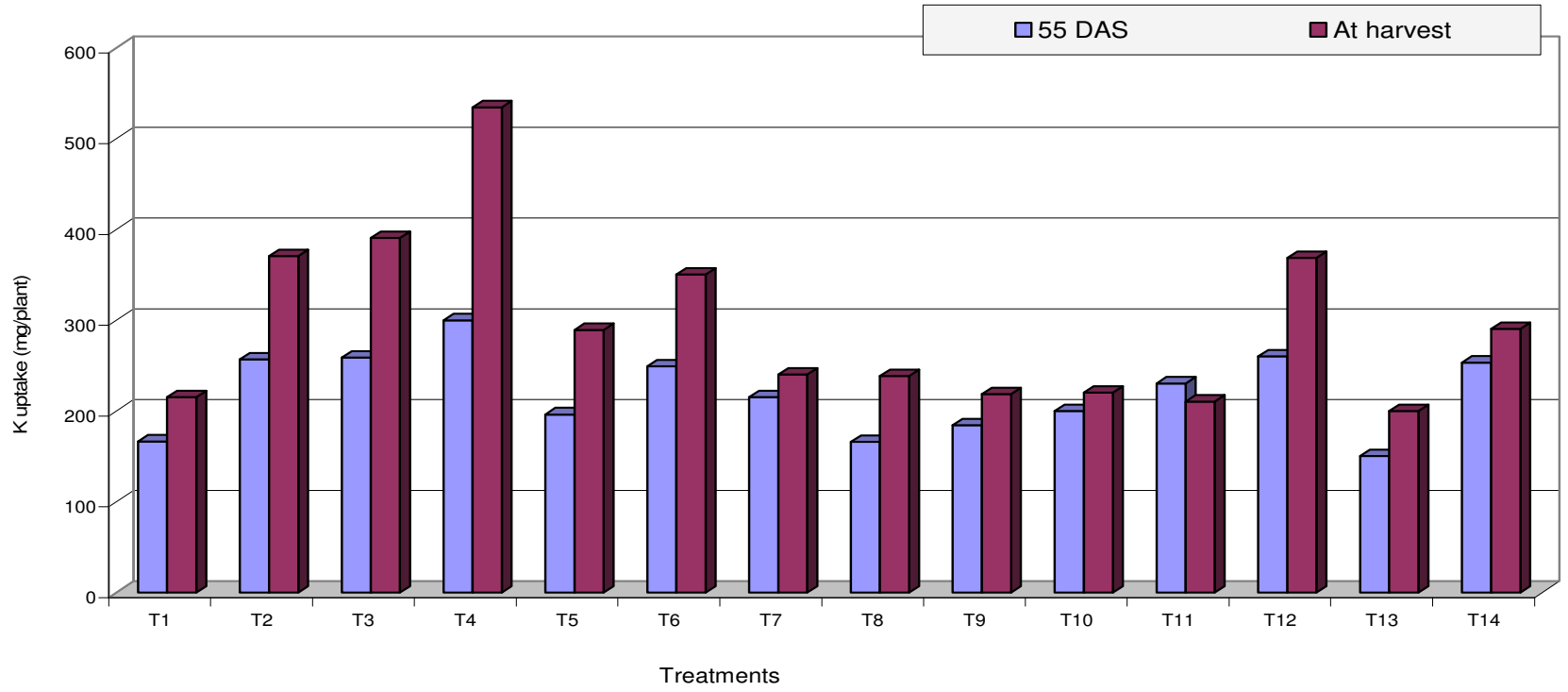


Fig. 5. Effect of efficient (KSB) potassium solubilizing bacteria on K uptake of maize plants at 55 DAS and at harvest

Fig. 5. Effect of efficient (KSB) potassium solubilizing bacteria on K uptake of maize plants at 55 DAS and at harvest

Among the inoculation treatments KSB 42 recorded maximum K uptake 260.33 mg/plant closely followed by KSB62 and KSB 11 (253.33 and 249.33 mg/plant, respectively) all of which were on par among themselves but significantly superior over all other inoculation treatments except the reference strain weight which they were on par. The KSB 42 (260.33 mg/plant) recorded significantly higher 'K' uptake over 25 per cent of RDK, whereas the KSB 62 and KSB 11 showed K uptake on par with 25 per cent RDK. The higher levels of 'K' 75 and 100 per cent RDK recorded maximum K uptake over all other treatments (259.00 and 300.00 mg/plant, respectively).

4.27.2 K uptake at harvest

The data on K uptake of maize plants at harvest as influenced by inoculation with KSB and K fertilizers are presented in Table 4.15.

Only six out of the 10 inoculated strains showed significantly increase in total K uptake over absolute control. Among the inoculation treatments KSB 42 recorded maximum total K uptake (369.67 mg/plant) which was significantly superior overall other strains except KSB 47, KSB35, KSB18 and KSB33 (200.00, 210.33, 218.67, 220.33 mg/plant) respectively, while the isolates KSB 42 and KSB 11 (368.67 and 350.33) respectively were on par with 25 per cent RDK. However, 75 and 100 per cent RDK recorded significantly higher total 'K' uptake (390.67 and 534.67 mg/plant) respectively over all other treatments.

5. DISCUSSION

Microorganisms play a key role in the field of agriculture by converting the unavailable form of nutrient to available form there by increasing its availability in soil and enhancing agricultural production.

Soils are the dynamic ecosystems that harbour many micro-organisms which are closely related to the plants. Microorganisms play their role in two ways one as pathogens causing diseases and the other as beneficial ones such as biological control agents and nutrient mobilizers and solubilizers. Our interest was to elucidate the mechanisms involved in solubilization of some of the fixed nutrients for the agriculture use.

Numerous microorganisms particularly those associated with roots have the ability to increase plant growth and productivity (Cheng *et al*., 1986). However, certain groups of microorganisms can directly or indirectly transform rocks and minerals in quantities large enough to influence the geological distributions. These transformations include enzymatic oxidation, reduction reactions, formation of chelates and complexes with protein, amino-acids, organic acids *etc*. (Henderson and Duff, 1963).

Plants absorb potassium in monovalent cation form soil solution. However, most of the potassium in soil is in the form of mineral deposits and not easily available for plant uptake. Even though high amount of potassium is present in the soil, it is not available to plants because of its presence in mineral bound form. It is reported that a variety of soil microorganisms have been found to solubilize silicate from insoluble mineral source, there by increasing availability to crop plants (Bunt and Rovira, 1955). Apart from potassium solubilization, these microorganisms are also able to solubilize phosphorus and produce plant growth promoting substance like auxins, giberllins which are known to stimulate the growth of crop plants (Sheng and Huang, 2001). The use of efficient K solubilizing microorganisms along with low grade rock potassium can enhance the potassium availability to crop plants (Han *et al*., 2006). Most of the research on microbial mineral potassium solubilization has been directed towards assessing the possible impact of these organisms on growth potassium uptake and yield of crop plants.

The mechanisms of mineral potassium solubilization has been a subject of study for a long time and is still a matter of curiosity. Although some work has been done on the biochemical and molecular mechanisms of mineral potassium solubilization by bacteria, no concerted efforts have been made in bacteria and other potassium solubilizers. It was in this context that investigations were carried out on the isolation, identification and characterization of soil bacteria and their ability to soluiblize insoluble potassic mineral, mechanism of solubilization, other beneficial traits and their influence on growth and nutrient uptake of maize plants. The results obtained on these investigations are discussed hereunder.

Isolation and characterization of potassium solubilizing bacteria from different crops

The chances of isolating microbial isolates for solubilization of insoluble mineral nutrients is more in the rhizosphere soils of many crops (Altamare, 1999). With this view different bacterial isolates were isolated from the rhizosphere soils of different crop plants from the places around Dharwad and Belgaum districts. It was interesting to note that ability of bacterial isolates from rhizosphere soils were able to grow and solubilize the medium containing fixed or insoluble from of nutrients.

The results are in agreement with the findings of Norkina and Pumpynaskaya (1956) who also isolated two strains of *Bacillus* sp. and *Pseudomonas* from rhizosphere soil of various crop plants as mineral potassium solubilizers.

In support of Gaur *et al.* (1973), Duff *et al.* (1963) also isolated 2-keto gluconic acid producing *Pseudomonas* strain capable of solubilizing many minerals like quartz, silicates and phlogopite. Similarly (Fredrich *et al*., 2004) isolated different groups of microorganisms like *Bacillus mucilaginosus* and *Thiobacillus thioxidans* capable of solubilizing silicates.

The results of this study strong by support the work of Christophe *et al.* (2006) who isolated many of the root associated bacteria contributing to mineral weathering. He demonstrated that *Burkholderia glathei* PMLI (12) significantly increased biotite weathering.

In the present study the results of all the experiments concludes that *Bacillus* sp. was potential in solubilizing the potassic mineral in this study. The results are in agreement with the Hu *et al.* (2006) who isolated two phosphate and potassium solubilizing *Bacillus* sp. from the soils, in the modified medium containing phosphorite and potassium minerals like kaolinite and potassium feldspar.

Characterization of bacterial isolates for release of mineral nutrients from their fixed source

Based on colony morphological and biochemical characters the organisms were tentatively identified as *Bacillus* and *Pseudomonas* sp. then the organisms were inoculated to broth medium containing fixed mineral sources. Similarly Fan and Yan (2006) also isolated and characterized bacteria *Bacillus mucilaginosus* capable of solubilizing two potassium bearing minerals like feldspar and illite. The probable mechanism of action as mentioned by these scientists for solubilization of potassium bearing minerals was by the action of organic acids like oxalic acid and capsular polysaccharides.

The morphological characterization revealed that all potassium solubilizing bacteria were gram positive short to long rods with spore production, but differed in their physiology and nutrition. Similarly, Avakyan *et al.* (1984), Webley *et al.* (1960) and Purushotham *et al.* (1974) reported *Bacillus mucilaginosus* solubilized insoluble silicates.

All the above mentioned reviewed will strongly support the present study carried out to isolate the bacterial strains like *Bacillus* and *Pseudomonas* sp. capable of solubilizing potassic mineral. Hence, these isolates were selected for further experiments.

All the thirty KSB strains were further screened for their ability to solubilize potassic mineral like muscovite mica in agar and broth medium. The zone of solubilization by all the mineral potassium solubilization strains ranges from 0.68 to 1.30 cm at 72 hours after incubation (HAI). Such observation were made earlier that among the K bearing silicate minerals mica was found to solubilize readily (Tandon and Sekhon, 1988; Sugumaran and Janarthanam, 2007; Mikhailouskaya and Tehernysh, 2005).

The potassium solubilizing bacteria were subsequently tested for the ability to release K from muscovite mica in the external broth. The amount of K released from muscovite mica ranged from 2.41 µg/ml to 44.49 µg/ml. Among the isolates *Bacillus* species KSB 11 showed the higher K release from the insoluble K source used. The findings are in agreement with the findings of Hu and Boyer (1996) who reported that *Bacillus megaterium* was capable of solubilizing mica in appreciable amounts.

The results also indicated great variation between the isolates to soluiblize the same or different source of insoluble potassium minerals (Mikhailouskaya and Tehernysh, 2005; Liu *et al*., 2006; Hu *et al*., 2006). Observed variation in the amount of potassium solubilization by the strains of same species of *Bacillus* and *Pseudomonas*. In contrast with the above Hu *et al.* (2006) reported that *Bacillus megatherium* and *B. mucilaginosus* were capable of solubilizing both rock phosphate and potassium. They also reported that co-inoculation of these two *Bacillus* species were potential in solubilizing potassium rocks. The present study thus indicated the *Pseudomonas* sp. was also capable of releasing some amount of potassium from mica (10.72 µg/ml) but was comparatively very less and the results compare well with the observations of (Badr, 2006). Greater release of K from muscovite have been documented by *Bacillus muciloginosus* (Sugumaran and Janarthanam, 2007).

Thus the ability of bacteria to release K largely depends on the nature of the potassium mineral compounds (Yakhontova *et al*., 1987). The variability among the bacteria indicates the importance of exploration of different mineral potassium solubilizing bacteria and understanding their solubilizing mechanisms.

The release of K from insoluble potassic mineral by the isolated bacteria increased with incubation time upto 20 days. The results also indicated variability in the amount of K released by different isolates. The differential efficiency of bacteria to solubilize insoluble inorganic potassium could be due to differences in their ability to release organic acids. The profiles of organic acid production by the bacteria revealed that oxalic acid and citric acid were the common organic acid produced by most of the isolates used in the study. The other organic acid produced include malic acid, succinic acid, tartaric acid. The production of

organic acids like oxalic acid, tartaric acid, citric acid, acetate by potassium solubilizing bacteria have been reported earlier by various workers (Sheng and He, 2006; Liu *et al*., 2006; Ullman *et al*., 1996; Vandevivere *et al*., 1994; Berthelin, 1983; Barker *et al*., 1998; Hazen *et al*., 1991; Barker *et al*., 1997; Friedrich *et al*., 1991 and Vainberg *et al*., 1980).

In addition to potassium soluiblization polysaccharide production could be another important desirable property of the K solubilizing microorganisms as it contributes an important fraction of the more stable soil humus (Mehta *et al*., 1961). It was in this contex that isolates were tested for production of polysaccharies. The results obtained showed wide variability in production of polysaccharide by K solubilizing bacteria. *Bacillus* sp. Strain KSB 11 and KSB 42 produced high amount of polysaccharides followed by KSB14, KSB16, KSB18, KSB33, KSB35 and KSB47. Similar observation on production of poysaccharides by KSB have been made by several workers (Sheng and He, 2006; Berthelin and Belgy, 1979; Malinovskaya *et al*., 1990; Liu *et al*., 2006; Grudev, 1987; Welch and Ullman, 1993).

Apart from solubilization of potassic minerals, many K-solubilizers are known to possess other beneficial properties like solubilization of insoluble inorganic phosphates, production of plant growth promoting substances and polysaccharide production *etc*.

The K solubilizing bacteria were also examined for solubilization of insoluble inorganic phosphates to release Pi from TCP in the external broth. All the isolates have shown the ability to release Pi from TCP at different incubation period the Pi released was maximum at 15 DAI ranged from 5.73 to 12.27 per cent. Similar observation of soluiblization insoluble phosphates by potassium solubilizing bacteria *Bacillus muciligenosus* reported earlier by Badr (2006) and Hu *et al.* (2006).

Potassium solubilizing bacteria were also examined for production of IAA and GA. All the isolates produce IAA and GA in the range of 1.10 to 16.50 µg/25 ml and 0.60 to 3.29 µg/25 ml broth respectively.

Sheng and Huang (2001) reported growth enhancement of *Bacillus* may also relate to its ability to produce hormones, especially IAA.

Based on the efficiency of K solubilization and P solubilization and PGPS production nine selected bacterial isolates were further examined for their performance to enhance growth, nutrient uptake and yield of maize plants. The results indicated in general that the inoculated bacteria increased the plant growth, nutrient uptake and yield components of maize plants over absolute control and 25 per cent of RDK control. The root length of maize plant was increased by 59.66, 55.00, 54.33 and 53.67 cm due to the inoculation of potassium soluiblizing bacterial strains KSB11, KSB62, KSB42 and reference strain respectively over 25 per cent RDK control.

Similarly increases in plant root length due to inoculation of *Bacillus edaphicus* potassium soluiblizing bacteria has been reported by Sheng and Hey (2006), maize (Wu *et al*., 2005), brinjal (Ramarethinam an dChandra, 2005), cotton and rape seed (Sheng, 2005).

Similar findings were also noticed by Berthelin and Leyval (1982) using the mycorrhizal fungi for the solubilization of mica. Finally, they have concluded that the weathering of biotite mica resulted in increase of potassium that had direct impact on maize.

In support of these findings Powell (1975) suggested that *Glomus mosseae* increased potassium uptake by non-symbiotic micoroganisms that stimulates growth by non-symbiotic microorganisms like *Azotobacter* and ultimately enhanced the plant growth. He also concluded that the non-symbiotic microorganisms present in rhizosphere would modify the chemical equilibrium of potassium solubility, so that the plant acts as potassium sink.

Similar results were obtained by Igual *et al.* (2001) who used phosphate solubilizing bacteria to increase potassium uptake by the test plants.

However, there is no experimental data on the ability of *Pseudomonas* sp. to solubilize mica and effect plant growth. Bacteria are found to posses solubilizing abilities. These mechanisms might account for at least some of these plant growth promoting effects and would provide new opportunities to study interactions with plants.

The plant height of maize plants was increased significantly due to inoculation of KSB strains over absolute control and 25 per cent RDK control but on par with 75 per cent RDK

control. An increase of 65.17, 65.28, 64.24 and 67.15 cm in the plant height of maize plants was noticed due to inoculation with potassium solubilizing strains KSB11, KSB62, KSB42 and reference strain respectively over 0 and 25 per cent RDK but on par with 75 per cent RDK control and are comparable with the results of Sheng and Hey (2006), Wu *et al.* (2005), Badar (2006) and Han and Lee (2005).

In accordance with the root and shoot growth and dry matter content in root and shoot as well as total dry matter content of maize plants was also enhanced due to the inoculation of KSB over absolute control 0 and 25 per cent RDK control. The total dry matter content of maize plants due to inoculation of KSB strains KSB 33 and KSB 11 was increased by 25.43 g and 28.33 g respectively over 25 per cent RDK control. The results are in agreement with those of Badr (2006) who obtained 58 per cent increased dry matter content of sorghum plants due to inoculation of silicate dissolving bacteria. Similarly results on the enhanced dry matter content of groundnut (Sugumaran and Janarthanam, 2007), wheat (Mikhailouskaya, 2005), brinjal (Nayak, 2001), chilli (Ramarethinam *et al*., 2005), tomato (Li, 2002), pepper and cucumber (Han *et al*., 2006).

The increased root and shoot dry matter yield of maize plants is evidenced by the enhanced root and shoot growth and enhanced K uptake by plants receiving KSB inoculants. The inoculation of KSB strains significantly increased the yield and yield parameters of maize plants over absolute control and on par with 25 per cent RDK control. The yield attributing characters like cob weight, grain yield and 100 seed weight of maize were significantly enhanced in the treatments receiving inoculation of KSB strains, KSB 11 and KSB 42. Further recorded an increase in the grain yield per plant by 51.37 g and 49.10 g over absolute control. Inoculation of potassium solubilizing bacteria *B. mucilaginosus* has been reported to significantly increase the yield of maize, sorghum, wheat, tomato, brinjal, cucumber and pepper, rice, cotton, chilli. Alexandrov (1958), Vintikova (1964), Muralikannan (1996), Kalaiselvi (1999), Zhang *et al.* (2004), Chandra *et al.* (2005), Supanjani *et al.* (2006).

The maize plants receiving KSB inoculation showed significantly higher K uptake over the absolute control. Among the inoculation treatments KSB42 recorded maximum K uptake 260.33 mg/pot followed by KSB62 and KSB11 (253.33 and 249.33 mg/pot respectively). All of which were on par among themselves but significantly superior over all other inoculation treatments. The KSB 42 recorded significantly higher K uptake over 25 per cent of RDK control (260 mg/pot) whereas the KSB 62 and KSB 11 showed K uptake on par with 25 per cent RDK. The higher levels of K 75 and 100 per cent RDK control recorded maximum K uptake over all other treatments (259.00 and 300.00 mg/pot respectively).

Similar results on the enhanced K uptake were obtained by Dattal *et al.* (1982), Lin (2002), Vessey (2003), Sheng *et al.* (2003), Zhang *et al.* (2004), Wu *et al.* (2005), Han and Wer (2005), Ramarethinam and Chandra (2005) and Sheng (2005).

Thus, the present study resulted in isolation and identification of a potassium solubilizing bacteria *Bacillus* species which proved better than the reference strain. The range of variability seen amongst isolates indicates that it is prudent and necessary to keep the isolation of beneficial bacteria a continuous programme. The additional beneficial traits exhibited by the strains indicate the possibility of isolating a strain with multiple beneficial effects. The strain *Bacillus* species KSB11, KSB42 and KSB62 can now be tested on the field to rate its performance as a biofertilizer.

6. SUMMARY AND CONCLUSIONS

Potassium availability to crop plants in soil is generally low since nearly 98 per cent of total K in soil is in mineral forms. Further fixation of added P in soil reduces the efficiency of applied K fertilizer since a large quantity become unavailable to plants.

Solubilization of soil mineral, by fungi and bacteria are well established. However, less information is available on K-solubilizing bacteria and their impact on growth and development of crop plants. In this context, attempts were made to isolate different K solubilizing bacteria from rhizosphere soil samples. The efficiency of the isolates to solubilize insoluble potassium mineral, production of plant growth promoting substance and other agronomically beneficial traits were studied under laboratory and pot culture condition. The *in vitro* efficient K-solubilizing bacteria were further tested for their effects on growth, nutrient uptake and yield of maize plants under green house condition.

The rhizosphere soil samples of different crops were used in the study for isolation of potassium solubilizing bacteria. A total of 30 KSB isolates are isolated on media supplemented with mica as a potassium source.

All bacterial cultures were identified upto genus level and were found to belong to the genera *Bacillus* and *Pseudomonas*. All the isolates were able to solubilize (mica) potassic mineral under *in vitro* condition. The amount of K released by the isolates ranged from 2.14 µg/ml to 44.49 µg/ml. Among the isolates KSB11 showed maximum solubilization (44.49 µg/ml) followed by KSB 42 (37.07 µg/ml).

All thirty isolates that showed mineral potassium solubilization activity were examined for the profile of organic acid production. They were found to produce one or the other organic acid oxalic acid and citric acid were the most common organic acids produced by all the isolates. Other beneficial traits like solubilization of insoluble phosphate and production of growth promoting substance were also studied.

The data on the solubilization of TCP under *in vitro* by the isolates, the amount of Pi released by the isolates from TCP ranged from 5.72 to 12.27 per cent. Among the isolates KSB 33 showed maximum release of Pi (12.27%) followed by KSB 35 (11.87%).

All the isolates were tested for production of plant growth promoting substances. The amount of IAA produced by the strains ranged from 1.10 to 16.50 and that of GA ranged from 0.60 to 3.29 µg/25 broth.

The analysis of spatial release of K by the isolates in general shown an increase in the amount of K released into mica broth with increasing period of incubation.

The results on polysaccharide production on mineral agar medium by the isolates indicated that three strains were able to produce high amount of polysaccharide, where as 6 isolates produced moderate amount of polysaccharide and all remaining isolates produced low polysaccharide.

Nine of these efficient K-solubilizing bacteria *Bacillus* species (KSB 11, KSB 14, KSB 16, KSB 18, KSB 33, KSB 35, KSB 42, KSB 47 and KSB 62) were further examined for their influence on the growth, nutrient uptake and yield of maize plants under green house condition. The results indicated that all the inoculated bacteria increased plant growth, nutrient uptake (K) and yield component of maize plants significantly over absolute control.

Among the strains KSB 11 followed by KSB 62 and KSB 42 performed best.

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APPENDIX I

Composition of media

Aleksandrov Medium (Hu *et al.,* 2006)

Glucose	-	5.0 g
Magnesium sulphate ($MgSO_4.7H_2O$)	-	0.005 g
$FeCl_3$	-	0.1 g
Calcium carbonate	-	2.0 g
Potassium mineral	-	2.0 g
Calcium phosphate	-	2.0 g
Distilled water	-	1000 ml

Starch agar (Eckford, 1927)

Peptone	-	5.0 g
Beef extract	-	3.0 g
Starch	-	10 ml
Distilled water	-	1000 ml
Agar	-	18 g
pH	-	7.2

Czapeck's Solution (Mahadevan and Sridhar, 1984)

$NaNO_3$	-	2.00 g
K_2HPO_4	-	1.00 g
$MgSO_4.7H_2O$	-	0.50 g
KCl	-	0.50 g
$FeSO_4.7H_2O$	-	0.01 g
Sucrose	-	30.00 g
Yeast extract	-	1.00 g
Distilled water	-	1000 ml

Nutrient gelatin (Cappuccino and Sherman, 1992)

Peptone	-	5.00 g
Beef extract	-	3.00 g
Gelatin	-	120.0 g
Distilled water	-	1000 ml
pH	-	6.8

Pikovskaya's medium (Pikovskaya, 1948)

Glucose	-	10.00 g
$MgSO_4.7H_2O$ (2.5%)	-	10.00 ml
$CaCl_2$ (1%)	-	10.00 ml
Tricalcium phosphate	-	5.00 g
Distilled water	-	1000 ml
pH	-	7.0
Agar	-	18.00 g

Skim milk agar

Skim milk powder	-	100.0 g
Peptone	-	5.0 g
Agar	-	15.0 g
pH	-	7.2

MR-VP broth (pH 6.9)

Peptone	-	7.0 g
Dextrose	-	5.0 g
Potassium phosphate	-	5.0 g

Luria agar

Tryptone	-	10.0 g
Yeast extract	-	5.0 g
Sodium chloride-	5.0 g	
Agar	-	18.0 g
Distilled water	-	1000 ml
pH	-	7.2

Salper's reagent

0.5M $FeCl_3$	-	1.0 ml
35% $HClO_4$	-	50 ml

Reagents for spectrophotometric estimation of GA

- Zinc acetate solution: 219 g of zinc acetate was dissolved in 80 ml of distilled water one ml of glacial acetate acid was added and the volume made up to 100 ml with distilled water.
- Potassium ferrocyanide solution: 16 g of potassium ferrolyanide solution in 100 ml of distilled water.

STUDIES ON POTASSIUM SOLUBILIZING BACTERIA

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ABSTRACT

Attempts were made to isolate potassium solubilizing bacteria from rhizosphere soil of different crops from Dharwad and Belgaum districts. A total of 30 bacteria isolates were tested for K solubilization and characterized upto genus level based on morphological and biochemical characters. The mechanisms involved in K soluiblization and other agronomical beneficial traits were also analyzed for selected efficient strains.

In vitro K solubilization by bacteria ranged from 2.41 µg/ml to 44.49 µg/ml. Oxalic acid, citric acid were the chief organic acids produced by the KSB isolates. All the isolates tested for other beneficial traits like solubilization of insoluble phosphate and production of growth promoting substance. The amount of Pi released by the isolates from TCP ranged from 5.72 to 12.27 per cent. The amount of IAA produced by the strains ranged from 1.10 to 16.50 and that of GA ranged from 0.60 to 3.29 µg/ 25 ml broth.

Nine efficient gram positive K soluiblizing bacteria were also examined for their influence on growth, K uptake and yield of maize plants under glass house condition. All the inoculated treatment with bacteria were found to increase growth parameters and yield components compare to absolute control and 25 per cent of RDK control *Bacillus* sp. KSB 11 recorded the highest yield (51.33 g/plant) and other parameters followed by KSB 62 and KSB 42. Three strains of present study *viz.,* KSB 11, KSB 62 and KSB 42 showed high potential among the KSB isolates. Thus it can be inferred that potassium solubilizing bacteria have the potential to use as bioinoculants.