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Impact of Potassium Solubilizing Bacteria on Growth and Yield of Mungebean *Vigna radiata*

KEYWORDS Potassium, rhizosphere, solubilization, mineralization

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ABSTRACT *Soil microorganisms are supportive in the transformation of soil potassium (K) and are thus an important component of the soil K cycle. These are effective in releasing K both from inorganic and organic pools of total soil K through their respective solubilizing and mineralizing abilities. To evaluate this, two promising organisms (KSB-1 and KSB-7) of capable of solubilization of both organic and inorganic potassium as investigated under in vitro conditions were evaluated in a pot trial for their rhizosphere activity and mineralization potential of organic K in soil, plant growth and yield. In response to inoculation with these selected potassium solubilizing bacteria (KSB), significant increases in seed germination, root and shoot length and number of leaves grain yield were observed which were increase to respectively, over uninoculated control in the presence of feldspar in Aleksandrov's agar medium. The study demonstrated that the use of KSB having multifaceted beneficial traits would be highly effective for improving growth and yield of crops.*

Introduction
The special focus on K solubilizer was due to the fact that potassium is one of the major nutrients required by all crops. It is a key element in many physiological and biochemical processes. 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, elite and orthoclases through production and excretion of organic acids (Fridrich *et al.,* 1991).

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 Potssium Solubilizing Bacteria (KSB) 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) are the major soil groups in the state. In general, black soils are high, red soils medium and lateritic soils lows 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 tons of K is being imported annually (Anonymous, 2003). 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 to study the influence of selected efficient mineral potassium solublizing bacteria on growth, of Mungbean (*Vigna radiata).*

Isolation and Screening of Potassium Solubilizers.
The collected soil samples from nearby the ceramic industries of the Kadi, North Gujarat Region. Soil samples were enriched with 2% insoluble potassium (feldspar), and incubated for 1week at room temperature. After adaptation, 1gm of soil was inoculated in Aleksandrov's broth and incubated at 37 ^{0}C on static condition for 1 week. 0.1 ml serial dilutions of enriched sample up to 10^{-5} were inoculated on Aleksandrov's agar medium (Hu *et al.,* 2006) (supplemented with 0.5 mg % Griseofulvin (antifungal antibiotic)) and incubated at 37 ^{0}C for 1 week, a selective medium for isolation of potassium solubilizing bacteria (Sugumaran and Janartham, 2007). Isolated colonies appearing in the 10^{-3}, 10^{-4} and 10^{-5} dilution plates of Aleksandrov's agar exhibiting cleared zone of potassium solubilization (indicating the ability to solubilize feldspar incorporated in the medium) were selected. Screening of these isolates was carried out by further inoculating the isolates in same Aleksandrov's agar medium and studying their ability of potassium solubilization by Khandeparkar's selection ratio (Gayal and Khandeparkar, 1979).

Based on the Khandeparkar's selection ratio, among the 7 bacterial isolates, 2 isolates exhibited the higher Potassium Solubilization (i.e. KSB-1 and KSB-7). Among the 7 isolates KSB-1 and KSB-7shows higher potassium solubilization values so these two isolates were selected for the further studies. KSB- 1 is Gram negative short rods while KSB-7 is Gram positive spore former and caplulated microorganism.both the isolates solubilize insoluble potassium from feldspar by organic acid production when studied by using Bromothymol Blue as pH indicator dye.

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 feldspar). One ml of overnight culture of each isolate was inoculated to 25 ml of GYF (Glucose Yeast Extract Feldspar) broth. All the inoculated flasks were incubated for two week at 28±2 ^{0}C. The amount of K released in the broth was estimated at day after days of incubation from flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 5,000 rpm for 10

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minutes in the cooling centrifuge 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 40 ppm KCl (Potassium Chloride) solution. The amount of potassium solubilized by the isolates was calculated from the standard curve. KSB-7 released maximum amount of K from feldspar 32.6 mg/lit followed by KSB-1 is 31.2 mg/lit.

Effect of Potassium Solubilizing Bacteria on Growth of Mungbean *(Vigna radiata)*

To study the effect of inoculation of 2 efficient isolates of Potassium solubilizing bacteria on growth, yield and nutrient content of Mungbean(*Vigna radiata*) plant. A pot culture experiment was conducted. Total three different experiments were planned. Experiment A: Seed application- seeds were treated with microbial inoculants for 30 minutes before sowing. Experiment B: Soil application - microbial inoculants were inoculated in soil. Experiment C: Seed + Soil application- seeds were treated with microbial inoculants for 30 minutes before sowing and microbial inoculants were inoculated in soil. The results were recorded for Seed germination , No. of leaves, Root length and shoot length.

Table.1 Experiment-A: Seed Application of K Solubilizing Microorganisms.

Treatment no.	Chemical Fertilizer K_2O kg/ha	Rock potassium K_2O kg/ha	Seed aapplication
T1	-	-	-
T2	20	-	-
T3	10	10	-
T4	10	10	KSB-1
T5	10	10	KSB-7
T6	10	10	KSB-1 + KSB-7

Fertilizer dose: All treatments (T2-T6) except absolute control (T1) received 40 kg K_2O / ha.

Seed Application:

KSB-1 → 3 ml containing 10^8 CFU / kg seed before sowing.

KSB-7 → 3 ml containing 10^8 CFU / kg seed before sowing.

Irrigation: As and when needed.

Table 2. Experiment-B: Soil Application of K Solubilizing Microorganisms.

Treatment no.	Chemical Fertilizer K_2O kg/ha	Rock potassium K_2O kg/ha	Soil Application of KSB
T1	-	-	-
T2	20	-	-
T3	10	10	-
T4	10	10	KSB-1
T5	10	10	KSB-7
T6	10	10	KSB-1 + KSB-7

Fertilizer dose: All treatments (T2-T6) except absolute control (T1) received 40 kg K_2O / ha.

Soil Application:

KSB - 1 → Apply @ 3ml containing 10^8 CFU/ pot

KSB - 7 → Apply @ 3ml containing 10^8 CFU/ pot

Irrigation: As and when needed.

Treatment no.	Chemical Fertilizer K_2O kg/ha	Rock potassium K_2O kg/ha	Seed Application	Soil Application
T1	-	-	-	-
T2	20	-	-	-
T3	10	10	-	-
T4	10	10	KSB-1	KSB-1
T5	10	10	KSB-7	KSB-7
T6	10	10	KSB-1 + KSB-7	KSB-1 + KSB-7

Table 3. Experiment-C: Seed + Soil Application of K Solubilizing Bacteria.

Fertilizer dose: All treatments (T2-T6) except absolute control (T1) received 40 kg K_2O / ha.

Seed Application:

KSB - 1 → Apply 3 ml containing 10_8 CFU / kg seed before sowing

KSB - 7→ Apply 3 ml containing 10_8 CFU / kg seed before sowing

Soil Application:

KSB - 1 → Apply @ 3ml containing 10_8 CFU/ pot

KSB - 7→ Apply @ 3ml containing 10_8 CFU/ pot

Irrigation: As and when needed.

Results and Discussion:

Colonically distinct total of 7 bacterial cultures were isolated exhibiting cleared zone of potassium solubilization (indicating the ability to solubilize feldspar incorporated in the medium) on Aleksandrov's agar and coded as KSB-1 to KSB-7. Based on the Khandeparkar's selection ratio, among the 7 bacterial isolates, 2 isolates exhibited the higher Potassium Solubilization (i.e. KSB-1 and KSB-7). Among them KSB -1(1.66 mm) and KSB-7 (1.44 mm) shows higher selection ratio of potassium solubilization. The amount of K released from feldspar in a broth by the isolates was studied at 7, 15, 20 days after incubation (DAI). The selected isolates exhibited cleared zone of potassium solubilization and yellow colour formation around the growth when grown on Aleksandrov's agar medium (supplemented with 2.5 mg Bromothymol blue (BTB)/100 ml) after the incubation at 30⁰C for 72 hrs. This yellow colour formation with the solubilization indicating that the solubilization is due to the acid production by the isolates. The amount of K released from mineral K by both bacterial isolates increased with increase in incubation time and was maximum at 20 DAI. The K released from feldspar by the bacterial isolates showed more than 25 mg/ml at 20 DAI. Among the isolates KSB-7 released maximum amount of K from feldspar 32.6 mg/ml Followed by KSB-1 31.2 mg/ml.

There is significant difference observed in the days required for the seed germination when the Mungbean plant was inoculated with the K solubilizing isolates. All the plants were showing seed germination at in 3-7 days after inoculation (Table-4). Among the inoculation treatments, increased number of leaves were recorded in both experiments A and C after 15 DAS i.e. the treatment of K solubilizing isolates and the soil plus seed treatment showed higher number of the leaves per plant to the single soil and seed treatment (Table-4). A significant increase was recorded in the shoot and root length of the Mungbean plant due to potassium solubilizing microorganisms inoculation treatments and in the seed and soil separate application. Maximum shoot length (11.5 cm) was recorded in treatment 6 (11.5 cm). While maximum Root length was recorded in the treatment 6 (i.e. KSB-1 plus KSB-7 treatment), with all the three application (6.4, 6.7 and 6.6 cm)

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Bacterial isolates showed significant increase in the shoot and root length (Table-5).

Table: 4. Effect of KSB-1 and KSB-7 isolates on Seed Germination and Number of Leaves to Mungbean Plant. The values are the mean of three replications (±SD).

S.N.	Treatments	Seed Germination (Days taken) (Mean± SD)			Number of Leaves per Plant at 15 DAS (Mean± SD) (in cm)		
		Exp A	Exp B	Exp C	Exp A	Exp B	Exp C
1	Uninoculated	7 ± 0	6.3 ±0.47	6.6 ± 0.47	4 ± 0.8	4 ± 0.81	3.6 ± 0.94
2	Uninoculated + soluble K	5.6 ± 0.47	5.3 ± 0.47	5.3 ± 0.47	4.3 ± 1.24	4 ± 0	5 ± 0.81
3	Uninoculated + Soluble K + Rock K	6 ± 0.81	4.3 ± 0.47	4.6 ± 0.47	4.6 ± 0.94	5.3 ± 0.47	6 ± 0
4	Soluble K + Rock K + KSB-1	4.3 ± 0.47	4.6 ± 0.47	4 ± 0.81	5.3 ± 0.47	5.3 ± 1.24	5.6 ± 0.94
5	Soluble K + Rock K + KSB-7	4 ± 0	3.6 ± 0.47	4 ± 0.81	6.6 ± 0.47	6.3 ± 0.47	6.6 ± 0.47
6	Soluble K + Rock K + KSB-1 + SB-7	4.6 ± 0.47	4 ± 0.81	3.6 ± 0.47	6.3 ± 0.94	6 ± 0.81	6 ± 0.81

Table 5. Effect of KSB-1 and KSB-7 isolates on Shoot and Root Length of Mungbean Plant. The values are the mean of three replications (±SD).

S.N.	Treatments	Root Length (Mean ± SD) 30 DAS			Shoot Length (Mean± SD) 30 DAS (in cm)		
		Exp A	Exp B	Exp C	Exp A	Exp B	Exp C
1	Uninoculated	5.3± 0.26	5.2± 0.12	5.6± 0.16	10.2± 0.21	9.9 ± 0.12	9.7 ± 0.12
2	Uninoculated + soluble K	5.7± 0.12	5.6± 0.08	5.4± 0.16	10.4± 0.04	10.3± 0.12	10.3 ± 0.20
3	Uninoculated + Soluble K + Rock K	5.4± 0.24	5.4± 0.29	5.8± 0.16	10.4± 0.04	10.3± 0.20	10.5 ± 0.26
4	Soluble K + Rock K + KSB-1	6 ± 0.08	6.2± 0.16	6.4± 0.33	10.8± 0.33	10.7± 0.24	10.7 ± 0.12
5	Soluble K + Rock K + KSB-7	6.2± 0.24	6.5± 0.33	6.3± 0.24	11.1± 0.16	11.1± 0.16	11.1 ± 0.24
6	Soluble K + Rock K + KSB-1 + KSB-7	6.4± 0.28	6.7± 0.16	6.6± 0.24	11.5± 0.32	11.3± 0.12	11.5 ± 0.12

Enterobacter hormaechei (KSB-8) when inoculated with the insoluble K (feldspar) increases the plant growth in terms of shoot & root length, shoot & root weight, plant height, total plant weight and dry weight of Okra (Prajapati *et al.*,2013).

Bertsch and Thomas *et al.* (1985) reported that most of the potassium in soil exists in the form of silicate minerals. The potassium can be available to plant when minerals are slowly weathered or solubilized. Our data agree with those already reported (Sheng 2005; Wu *et al*. 2005). 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.

Ramarethinam and Chandra (2006) studied the effect of KSB/KMB, *F. aurentia* (symbion liquid - K formulation) on Brinjal (*S. melongena* L.). Different treatments involving KSB/KMB, *F. aurentia* and potash fertilizer as standalone treatment and combination treatments were carried out and a significantly high yield, plant height, root length, potassium uptake and chlorophyll content as compared to the control.. 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

Conclusion:

The K rich soil samples nearby ceramics industries were used in the study for isolation of potassium solubilizing bacteria. A total of 7 KSB isolates are isolated on media supplemented with feldspar as a potassium source. All the isolates were able to solubilize (feldspar) potassic mineral under *in vitro* condition. The amount of K released by the isolates showed more than 25 mg/ml at 20 DAI. Among the isolates KSB7 showed maximum solubilization (32.6 mg/ml) followed by KSB-1 (31.2 mg/ml). On the basis of colonical, morphological and biochemical characterization KSB-1 is Gram Negative strain while KSB-7 is Gram Positive *Bacillus sp.* Both of efficient K-solubilizing bacteria (KSB-1 and KSB-7) were further examined for their influence on the growth and nutrient uptake of Mungbean plants. The results indicated that all the inoculated bacteria increased plant growth and nutrient uptake (K) component of Mungbean plants significantly over absolute control.

REFERENCE

1. Aleksandrov, V. G., Blagodyr, R. N. and Iiiev, I. P. (1967). "Liberation of phosphoric acid from apatite by silicate bacteria." Mikrobiyol Zh. (Kiev), 29: 111-114. 2. Anonymous, (2003). "Agricultural Statistics at a Glance, Ministry of Agriculture Cooperation", New Delhi, pp.51-53. 3. Bertsch, P. M. and Thomas, G. W. (1985). "Potassium status of temperature region soils." In: Munson, R. D. (Ed.) Potassium in agriculture ASA, CSSA and SSSP, Madison, WI, pp.131-162. 4. Friedrich, S. N. P., Platonova, G. I., Karavaiko, E., Stichel and Glombitza, F. (1991). "Chemical and microbiological soluiblization of silicates." Acta. Biotech, 11: 187-196. 5. Gayal, S. and Khandeparker, V. G. (1979). "Production of Amylase by Bacillus subtillis." Indian journal of Microbiology, 9: 226-231. 6. Hu, X. F., Chen, J. and Guo, J. F. (2006). "Two phosphate and potassium solubilizing bacteria isolated from Tiannu mountain, Zhejiang, China." World J. Micro. Biotech, 22: 983-990. 7. Prajapati, K., Sharma, M. C. and Modi, H. A. (2013). "Growth promoting effect of potassium solubilizing microorganisms on okra (Abelmoscus esculantus)" International Journal of Agricultural Sciences and Research, 3(1): 181-188. 8. Rajan, S. S. S., Watkinson, J. H. and Sinclair, A. G. (1996). "Phosphate rock of for direct application to soils." Adv. Agron., 57: 77-159. 9. Ramarethinam, S. and Chandra, K. (2005). "Studies on the effect of potash solubilizing/mobilizing bacteria Frateuria aurantia on brinjal growth and yield." Pestol., 11: 35-39. 10. Sheng, X. F. (2005). "Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of bacillus edaphicus." Soil Bio. Biochem., 37(1): 1918-1922. 11. Sheng, X. F. and He, L. Y. (2006). "Solubilization of potassium bearing minerals by a wild type strain of Bacillus edaphicus and its mutants and increased potassium uptake by wheat." Canadian J. Microbiol., 52(1): 66-72. 12. Sperberg, J. I. (1958). "The incidence of apatite solubilizing organisms in the rhizosphere and soil." Australian J. Agril. Resou. Econ., 9: 778. 13. Sugumaran, P. and Janarthanam, B. (2007). "Solubilization of potassium containing minerals by bacteria and their effect on plant growth." World J. Agric. Sci., 3(3): 350-355. 14. Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C. and Wong, M. H. (2005). "Effect of biofertilizer containing N-fixer, P and K soluiblizers and AM-fungi on maize growth." Geoderma, 125: 155-166. 15. Zhang, C. J., Tu, G. Q. and Cheng, C. J. (2004). "Study on potassium dissolving ability of silicate bacteria." Shaguan College Journal, 26: 1209-1216.