ISOLATION AND CHARACTERIZATION OF POTASSIUM SOLUBILISING MICROORGANISMS FROM SOUTH GUJARAT REGION AND THEIR EFFECTS ON WHEAT PLANT

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ABSTRACT

Potassium is the third most important plant macronutrient after nitrogen and phosphorous. The potassium is a fundamental macronutrient for plant growth and plays important roles in making active of several metabolic processes including protein synthesis, photosynthesis, and enzyme activation. The rhizosphere microbes play a significant role in solubilizing the unavailable form of potassium and make available to the plant roots. Most of the work has been done on nitrogen fixing and phosphate-solubilizing microbes, so the objectives of this study were to isolate and characterize of potassium solubilizing microorganisms from south Gujarat region and their effects on wheat seedling. All the isolates were tested for their enzymes production, plant growth promoting activities like phosphate solubilization, nitrate reduction, ammonia production and HCN production, phytohormones productions namely gibberellins and IAA ability and were tested for their plants growth promotion activity by performing seed germination assay. Five potassium solubilizing bacteria were isolated from rhizospheric soil coded as KSB1, KSB2, KSB3, KSB4, KSB5 were capable of solubilizing insoluble potassium. From all the five isolates tested, one isolate KSB2 was able to produce amylase, gelatinase and lipase enzymes and one isolate coded as KSB3 was found to be negative for any of those enzymes. Only one isolate KSB2 have shown the ability of free nitrogen utilization but rest all isolates were able to solubilize phosphate and unable to produce HCN and gave negative test. All the isolates were able to produce gibberellins plant hormones and all were unable to produce IAA plant hormones. The current study reveals that, the inoculation of potassium solubilizing isolates, potentially increase the root and shoot lengths of seedling by performing the seed germination assay. It is well known that the application of KSB can be a promising technique to solubilize the K reserves from soil and make it available to the plants, resulting in promotion of plant growth and minimizing the application of K fertilizers.

Keywords: Potassium solubilizers, gibberellins, Indole acetic acid, seed germination assay, biofertilizers

INTRODUCTION

Chemical fertilizers have aided farmers in increasing crop production since the 1930's while chemical fertilizers have their place increasing plant nutrients in adverse weather conditions or during times when plants need additional nutrients, there are also several harmful effects of chemical fertilizers. Chemical fertilizers may cause include waterway pollution, chemical burn to crops, increased air pollution, acidification of soil and mineral depletion of the soil. Chemical fertilizers are high in nutrient content such as nitrogen (https://www.hunker.com).

After nitrogen (N) and phosphorous (P), potassium (K) is the most important plant nutrient that has a key role in the growth metabolism and development of plants. In addition to increasing plant resistance to diseases, pests, and abiotic stresses, K is required to activate different enzymes responsible for plant and animal processes such as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation. K is present in several forms in the soil, including mineral K, non-exchangeable K, exchangeable K, and solution K. depending on the soil type, from 90 to 98% of soil K is mineral L and most of this K is unavailable for plant uptake. Silicate minerals such as K-feldspars and biotitic are the most common minerals in the earth's crust and are source of inorganic nutrient in soils to provide optimal nutrition for crops (Gallegos-Cedillo *et. al.*, 2016).

K-solubilizing microorganisms have been isolated from rhizosphere soil of different crops, which cause potassium solubilization by production of polysaccharides. These potassium solubilizing bacteria (KSB) could be applied as a potential biofertilizers along with application of rock K minerals to provide a continuous supply of available potassium for increasing the crop yield (Sindhu *et. al.*, 2016). The present study was aimed to isolate and characterized potassium solubilizing microorganisms from south Gujarat region and their effects on wheat plants.

METHODOLOGY

Sample collection:

Rhizospheric soils of *Cajanus cajan*, *Trigonella foenum-graecum*, *Oryza sativa* and *Triticum aestivum* were collected from the depth of 9 cm from different region of Tithal village, Gujarat. Soil samples were then packed in polythene bags and processed.

Isolation of rhizospheric microorganisms:

An aliquot (0.1 ml) of the bacterial suspension was spreaded on the plates of Tryptic soya Agar (TSA) medium. Plates were incubated at 28°C for 2-3 days (United States Pharmacopeial Convection 2001).

Screening of Potassium solubilizing microorganisms (KSM):

Screening was carried out using spot assay. Isolates were placed on sterile modified Aleksandrow agar medium supplemented with insoluble potassium aluminium silicate and incubated at room temperature for 3-4 days and were observed for potassium solubilizing colonies (Rajawat *et. al.*, 2016).

Plants growth promoting activities:

Potassium solubilizing microorganisms were isolated and were further processed for their plants growth promoting activities. Standard protocols were followed for the estimation of phosphate solubilization, nitrate reduction, ammonia production, HCN production, and also phytohormones like IAA and Gibberellin production.

- **Phosphate solubilization test:**

  Pikovskaya's agar medium was used for the phosphate solubilizing test. Isolates were checked for their ability to solubilizing phosphate by spot inoculation on Pikovskaya's agar medium plates and was then incubated at 25˚- 28˚C for 2-3 days. Clear zone around the bacterial colony indicates phosphate solubilizing ability of microorganisms (Kumar, 2016).

- **Nitrate reduction test :**

  Peptone nitrate broth (PNB) was inoculated with a loopful of test culture and incubated the medium at 37°C for 24 hours. The reagent A (α-napthylamine) and B (sulphanilic acid) were added in order. The development of red color within 30 seconds after adding test reagents was observed (Nawadkar *et. al.,* 2015).

- **Ammonia production test:**

  4% peptone broth were inoculated with isolates and incubated at 37°C for 24 hours. After incubation Nessler's reagent was added. Formation of yellow to brown precipitate showed the presence of ammonia (Cappuccino and Sherman, 1992).

- **HCN production test:**

  Nutrient agar plate containing 4.4g glycine/l were inoculated with isolates and then a whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution were placed in the top of plates. Plates were sealed with parafilm and incubated at 28°C for 4 days. Development of orange to red colour indicated HCN production (Lorck, 1948).

- **Test for Gibberellins content:**

  Gibberellic acid was measured using the standard method of borrow isolates were grown on nutrient broth medium and incubated at room temperature for 7 days. After that broth was centrifuged for 10 minutes at a speed of 8000 rpm. The culture was put into 15 ml reaction tube and added 2 ml of zinc acetate solution. Subsequently, a 2 ml of potassium ferrocyanide solution was added and centrifuged for 10 minutes at 8000 rpm. A total of 5 ml of supernatant was added 5 ml of 30% hydrochloric acid and incubated at 27°C for 75 minutes. The measurement of absorbance used a spectrophotometer at 254 nm (Gusmiaty *et. al.*, 2019).

- **Test for the Indole acetic acid (IAA) content:**

  Isolates were grown on 100 ml of liquid nutrient broth medium and incubated at room temperature in dark conditions for 5 days. A 1.5 ml bacterial culture was centrifuged for 10 minutes at speed of 8000 rpm. 1 ml the supernatant was added with 4 ml of Salkowski reagent (150 ml H_2SO_4, 250 ml sterile aquades and 7.5 ml $FeCl_3$ $6H_2O$ 0.5 M) and the solution mixture was incubated in the dark room for 24 hours at room temperature under dark conditions. Measurement of IAA isolates used spectrophotometer at 520 nm wavelength (Gusmiaty *et. al.*, 2019).

Seed germination assay

Wheat (*Triticum aestivum*) seed germination was done by applying the culture of potassium solubilizing isolates obtained. The isolates were grown in sterile Tryptic soya broth. Seeds of *Triticum aestivum* were surface sterilized with 0.1% HCl_2 for 3 minutes washing with distilled water for 6 times. The surface sterilized seeds of *Triticum aestivum* were bacterized with inoculums for 30 minutes and were then transferred on moist sterilized filter paper in Petri plate and were incubated at room temperature & left undisrupted. Seed soaked in sterile distilled water were used as negative control. The specified distilled water addition was followed every day until 7^{th} day. The root and shoot length were measured (Rodge *et. al*., 2016).

RESULTS AND DISCUSSION

Rhizospheres of *Cajanus cajan*, *Trigonella foenum-graecum, Oryza sativa* and *Triticum aestivum* were collected from the depth of 9 cm from different locations of Tithal village The present investigation was carried out at the department of microbiology at Dolat-Usha Institute of Applied Science Valsad, Gujarat. In the present study, 21 different types rhizospheric microorganisms were collected on Tryptic soya agar medium (TSA). All the collected samples were screened for potassium solubilizing activity, wherein 5 isolates were obtained which was able to solubilize potassium. In the current study, the obtained 5 potassium solubilizing isolates have found to solubilize insoluble potassium source and gave yellow colour zone around the colonies (Figure 1). All the 5 Potassium solubilizers were further studied and characterized morphologically and by performing various biochemical tests.

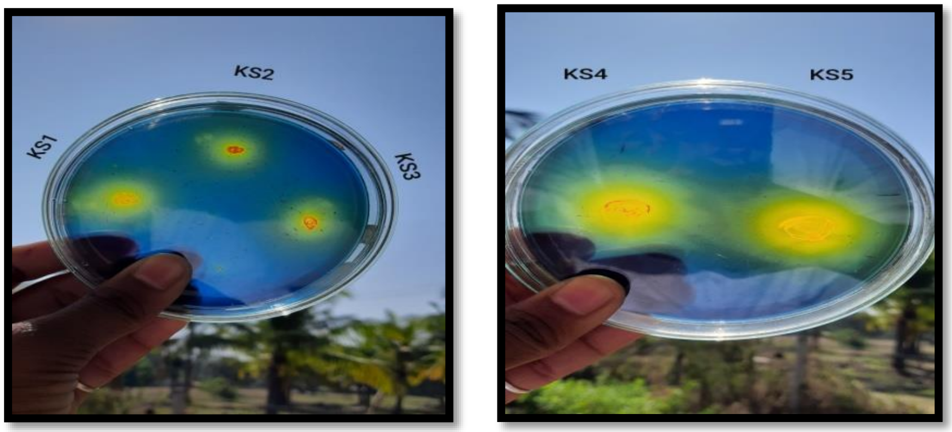


Figure 1: Potassium solubilization on modified Aleksandrow agar plate supplemented with potassium aluminium silicate

In 2015, Shree *et. al.,* in their studies, has isolated four potassium solubilizing bacteria which have solubilised insoluble potassium source in to soluble form and gave yellow zone around the colonies tested on Aleksandrow agar plates. The similar work was also done by Rajawat *et. al.,* (2016), they suggested a modified plate assay for rapid screening of KSB, based on improved visualization of halo zone formation around the colonies on agar plates, through inclusion of an acid-base indicator dye bromothymol blue (BTB), to modify the Aleksandrow medium. In 2018, Fatharani and Rahayu have also studied and isolated seven bacterial isolates which were capable of solubilizing potassium.

Results of morphological and biochemical characterization

In the present study, all the isolates were studied for their colonial characteristics and were reported with differed size, shape, opacity, surface and many with each other. From all isolates some isolates were medium or large in size, some were small and some were pin point. Most of the isolates were having round shape accept two isolates which were having rough and irregular (KS1 and KS2) shape. All having smooth surfaces accept two KS1 and KS2 having irregular and erose margin. All isolates were translucent accept one KS2 which was opaque. Only one isolate KSB1 was having green pigmentation rest of all were non-pigmented. Most of them were flat and some were raised. The same work was done by Shree *et. al.*, 2015, who were isolated four bacteria which all were round in shape and two were irregular. All were having different pigmentation like white, yellow and creamy white. Two isolates were flat and other two were convex in elevation. All isolates were having different size like some were large and some small. From four isolates, two were having entire margin and other having undulated.

Table 1: Morphological characteristics of potassium solubilizers

CHARACTERISTIC	ISOLATES				
	KSB1	**KSB2**	**KSB3**	**KSB4**	**KSB5**
GRAM'S STAINING	Gram Negative Rods	Gram Negative Rods	Gram negative Rods	Gram negative Rods	Gram Negative Rods
CAPSULE STAINING	Non Capsulated	Capsulated	Non Capsulated	Non Capsulated	Non Capsulated
MOTILITY TEST	Motile	Motile	Motile	Motile	Motile

In the present study, all the potassium solubilizing isolates were characterized morphologically by performing staining techniques and biochemically by performing various tests. Gram reactions of potassium solubilizing microorganisms are shown in Table 1 where all potassium solubilizing microorganisms were found bacteria and thus coded as KSB1, KSB2, KSB3, KSB4 and KSB5. All isolates found were Gram negative and rod shaped bacteria and one was capsulated which was KSB2 and rest were non capsulated and all were motile.

In 2015, Shree *et. al.*, has isolated four bacteria coded as NKC-13, NKC-20, NKC-28 and NKC-35 and performed Gram reactions for further identification of isolates. In their studies, they identified two isolates (NKC-13 and NKC-20) were Gram negative and other two were Gram positive. Out four isolates, only one isolate was non-motile and rests of all were motile.

Potassium solubilization by rhizobacterial isolates was studied on Aleksandrow agar plates. Total 5 bacteria from the rhizosphere were able to solubilize potassium aluminum silicate in the medium. Biochemical characterizations of all five potassium solubilizers were examined by different biochemical tests which are shown in Table 2.

In the current study, all the isolates were found negative for Indole production test and urea hydrolysis test. KSB3 was found positive for M-R and V-P tests and rest were negative for M-R and V-P test. All the isolates were found positive for ammonia production test which was another important trait of PGPR that indirectly influence the growth of plants. The similar work was also carried out by Shree *et. al.* in 2015.

Table 2: Biochemical characterization of potassium solubilizers

Test /Isolates	KSB1	KSB2	KSB3	KSB4	KSB5
Indole test	-	-	-	-	-
M-R test	-	-	+	-	-
V-P Test	-	-	+	-	-
Citrate utilization	+	+	+	+	+
Urea hydrolysis	-	-	-	-	-
Nitrate reduction	-	-	+	+	+
Ammonia production	+	+	+	+	+
Catalase test	+	+	+	+	+
Oxidase test	+	+	-	+	+

Results of Enzymatic activity

In the current study, all the five isolates were tested for their enzymatic activities by performing starch, gelatine and lipid hydrolysis tests. From all the five isolates, one isolate coded as KSB2 was found to be positive for all the three tests and was able to produce amylase, gelatinase and lipase enzymes and thus was able to hydrolyse starch, gelatine and lipid respectively. On the contrary, on isolate coded as KSB3 was found to be negative for all the tests performed and unable to produce any of those enzymes (Figure 2, Table 3). The similar work was done in 2015 by Shree *et. al.*, in their studies, they have isolated four isolates which had solubilised potassium and were also hydrolysed starch by producing amylase enzyme and utilized gelatine by producing gelatinase enzyme.

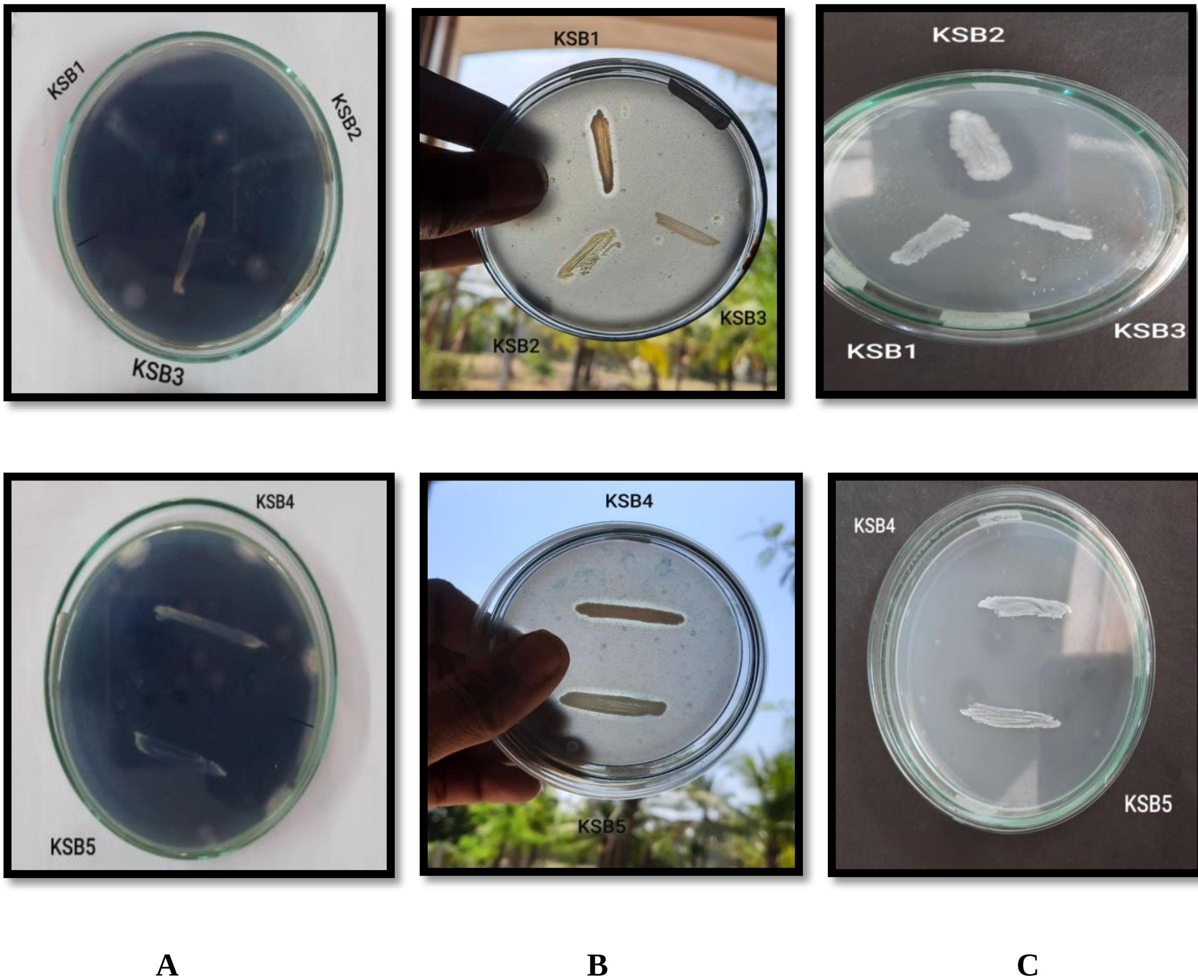


Figure 2: Results of starch hydrolysis (A), lipid hydrolysis (B) and gelatine hydrolysis (C) by potassium solubilizers

Table 3: Enzymatic activity of isolates

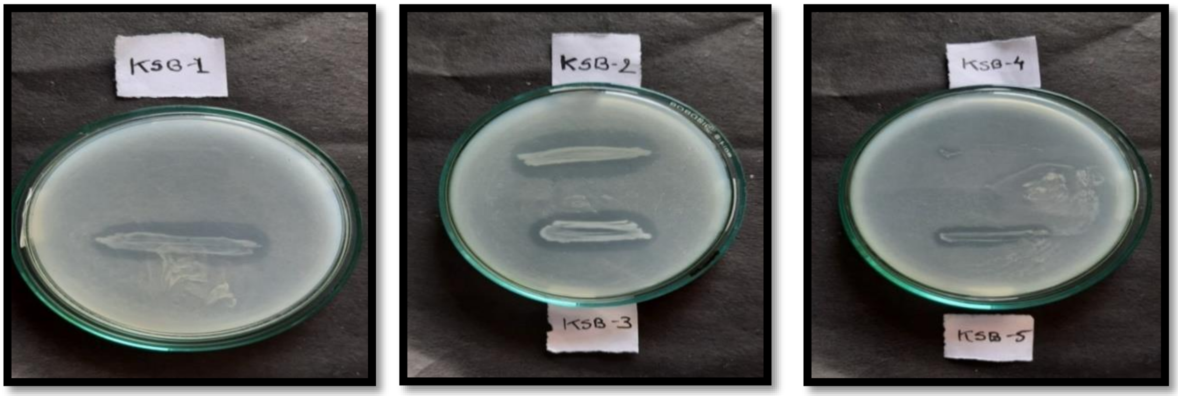
Test	Potassium solubilizers				
	KSB1	KSB2	KSB3	KSB4	KSB5
Starch hydrolysis	+	+	-	-	-
Gelatine hydrolysis	-	+	-	-	-
Lipid hydrolysis	+	+	-	+	+

Table 4: Plant growth promoting activity of isolates

Test	Potassium solubilizing organisms				
	KSB1	KSB2	KSB3	KSB4	KSB5
Nitrogen fixation	-	+	-	-	-
Phosphate solubilization	+	+	+	+	+
HCN production	-	-	-	-	-

In Table 4, plant growth promoting activities of isolates are shown which are nitrogen fixation, phosphate solubilization and HCN production. Microorganisms play important role in agriculture. They transform unavailable form of nutrient to available form in soil thereby increasing its availability to crops that enhance agricultural production. In the present study, only one isolate KSB2 having ability to utilize free nitrogen by using Ashby's mannitol agar medium. All isolates were able to solubilize phosphate and give clear zone around the line of inoculation. All isolates were not able to produce HCN and gave negative test. In figure 3 images of nitrogen fixation and phosphate solubilization are shown.

A



B

Figure 3: Results of free nitrogen fixation (A) and phosphate solubilization (B) activities by potassium solubilizers

Plant growth promoting activity

Table 5: Gibberellins and IAA production ability of potassium solubilizers

Isolates	Gibberellins	IAA
	OD at 245nm	OD at 520nm
KSB1	0.021	-
KSB2	0.074	-
KSB3	0.111	-
KSB4	0.133	-
KSB5	**0.430**	-

PGPR strains use one or more direct or indirect mechanisms to enhance growth and health of plants. Although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of mineral nutrient uptake are usually believed to be involved in plant growth promotion (Glick 1995). In Table 5, results of phytohormones are shown in which all the isolates were able to produce gibberellins plant hormones and all were unable to produce IAA plant hormones. In gibberellins production highest production was detected by KSB5 which is 0.430 at 245 nm wavelength.

Effects of potassium solubilizers on root and shoot lengths of wheat seedlings

Isolates were tested for their plant growth promotion by inoculating wheat seeds with the isolates and incubated at room temperature for 7 days and observed for their shoots and roots length. (Rodge *et. al*., 2016). In the present study, seed germination was performed with wheat seeds and results were reported after 7 days of incubation. The maximum length of shoot obtained was 4 cm and root was 5.5 cm which was obtained by KSB1 isolate and minimum growth seen in KSB2 (Figure 4 and 5).

Figure 4: The root and shoot growth of the wheat seeds bacterized with K solubilizing isolates

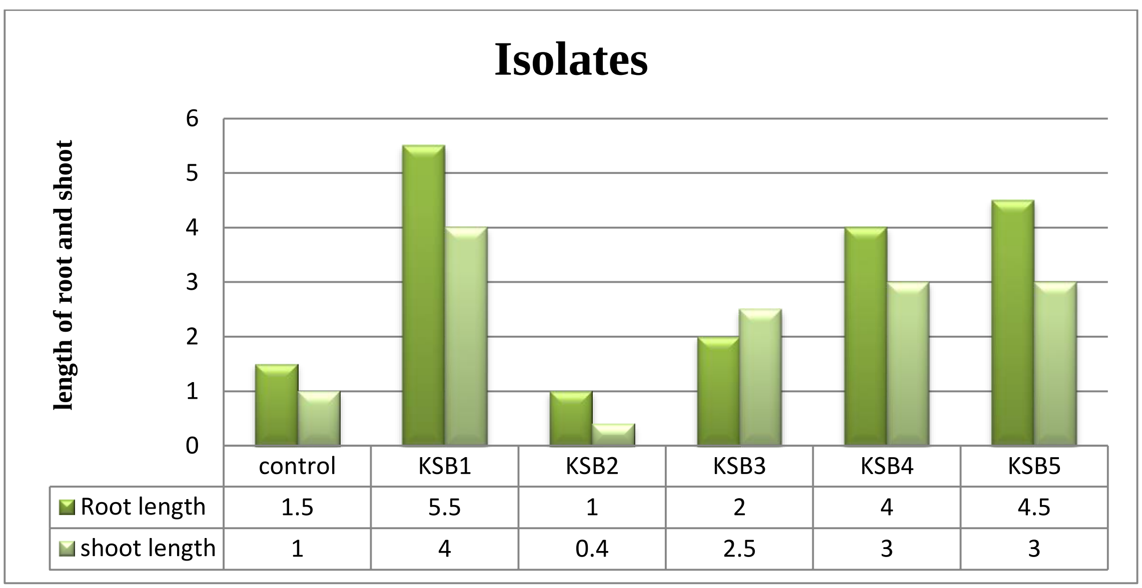


Figure 5: Graphical representation showing effects of potassium solubilizers on root and shoot lengths of wheat seedlings

In the present study, control which was devoid of any culture, all seeds bacterized by the isolates had flourished very well. However, comparing amongst the bacterized seeds, maximum growth was observed in the seeds bacterized with KSB1, which shows its ability and efficiency to be act as biofertilizer. Thus, the current study revealed that the KSB can be used in agriculture field for the plant growth promotion because it promotes the growth of plants by solubilizing inorganic potassium minerals. Rhizospheric bacteria, particularly beneficial bacteria can improve plant performances under environmental stress and consequently enhance the yield. The major limitation to a more widespread use of seed inoculation has generally been the variability in effects in both field and laboratory studies (Nadeem *et. al.,* 2014).

CONCLUSION

In the present study, five isolates were obtained and screened for their ability for potassium solubilization from rhizospheric soils of *Cajanus cajan, Trigonella foenum-graecum, Oryza sativa* and *Triticum aestivu.* All isolates were grown and tested on potassium aluminium silicate containing Aleksandrow agar plate and were characterized morphologically and biochemically, where all were found bacteria. From all the five isolates tested, one isolate KSB2 was able to produce amylase, gelatinase and lipase enzymes. On the contrary, one isolate coded as KSB3 was found to be negative for all the tests performed and unable to produce any of those enzymes. Only one isolate KSB2 have shown the ability of free nitrogen utilization but rest all isolates were able to solubilize phosphate and unable to produce HCN and gave negative test. All the isolates were also tested for phytohormones production where all were able to produce gibberellins plant hormones and all were unable to produce IAA plant hormones. The current study reveals that, the inoculation of potassium solubilizing isolates, potentially increase the root and shoot lengths of seedling by performing the seed germination assay. It is well known that the application of KSB can be a promising technique to solubilize the K reserves from soil and make it available to the plants, resulting in promotion of plant growth and minimizing the application of K fertilizers.

CONFLICTS OF INTEREST:

The authors declare that there are no conflicts of interest.

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