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Effects of *Serratia liquefaciens* isolate KM2 on Physiology and Phytohormones of Mung bean (*Vigna radiata* L.)

Meha D. Desai[1], K. P. Suthar[2*] and M. D. Khunt[3]

[1]Department of Biotechnology, Veer Narmad South Gujarat University, Surat 375 007, Gujarat, India

[2]Department of Plant Molecular Biology and Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari 396 450, Gujarat, India

[3]Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari 396 450, Gujarat, India

*Corresponding Author E-mail: kiransuthar@nau.in

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ABSTRACT

The potential Plant Growth Promoting Rhizobia Serratia liquefaciens isolate KM2 (SL-KM2) having direct and indirect PGPR traits was evaluated for its effect on growth and biochemical content of Mung bean (var. GM2). The SL-KM2 seed treatment improved the physiological parameters viz., root length, root volume, shoot length and fresh biomass as compared to untreated control. The maximum root length (22.5 cm), root volume (1.513 ml), shoot length (8.1 cm) and fresh biomass (0.48 g) was observed under seed treatment condition at 10 DAS, further these physiological parameters was improved as compared to untreated at 20 DAS. The seed treatment also improved the biochemical content at 10 and 20 DAS, where maximum cholorphyll (0.76 mg g^{-1}), protein (1.63 %) and total phenol content (leaves: 0.23%; Roots: 0.24%) was reported at 20 DAS. The seed treatment positively influences phytohormones content where the increase in gibberellic acid (GA_3) and indole acetic (IAA) content in leaves and root tissue was observed whereas jasmonic acid (JA) and abscisic acid (ABA) content was reduced. The maximum increase in GA_3 (64.56 nM g^{-1} FW) and IAA (15.13 nM g^{-1} FW) was observed under SL-KM2 seed treatment condition at 20 DAS. Overall, SL-KM2 seed treatment improved Mung bean growth, biochemical content and positively influenced the phytohormones that lead to improved plant development. This isolate can be in future exploited under field condition.

Key words: *Mung bean (Vigna radiata L.), Serratia liquefaciens isolate KM2, PGPR, Phytohormones*

INTRODUCTION

Mung bean (*Vigna radiata* L.) is an important pulses crop originated from India. Mung bean, similar to other pulses, is grown primarily for its protein rich seeds (20-25%). It is also high source of fiber, antioxidants and phyto-nutrients and low in fat and calories[1]. Over the last few decades, the agriculture policy in India has undergone a major change though there is a remote possibility of increasing area under pulses.

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Alternatively, short duration pulse may be grown during turnover period between rice-wheat cropping system. However, Mung bean productivity is influenced by nutrient supply, agronomic practices, further various abiotic (heat, cold, salinity, etc.) and biotic (diseases, insects, etc.) stresses[2]. In modern cultivation process indiscriminate use of fertilizers, particularly the nitrogenous and phosphorus, has led to substantial pollution of soil, air and water. Excessive use of these chemicals exerts deleterious effects on soil microorganism, affects the fertility status of soil and also pollutes environment leads to reduction in pH and exchangeable bases thus making them unavailable to crops and the productivity of crop decline[2].

For sustainable agriculture and to reduce environmental hazard microorganism provide an attractive alternatives in the form of bio-fertilizers and bio-pesticides. Rhizosphere zone comprise of environment of microorganisms around plant root is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria leads to higher bacterial population in this zone[3].

These microorganisms can effect plant growth and reduce chemical fertilizers application and environmentally beneficial low cost alternative. Use of plant growth promoting microorganisms (PGPRs) is a well recognized soil and crop management practices to achieve more sustainable agriculture and to improve fertility of soil[4]. The PGPRs have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen transferred to the plant[5], production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones. The different PGPR strains have been reported to improve plant growth, development and ultimately lead to higher production[6,7,8,9]. So, in present study the effect of *Serratia liquefaciens* isolate KM2 (*SL*-KM2) having direct and indirect PGPR traits was evaluated on physiology and biochemical content of Mung bean.

MATERIALS AND METHODS

Plant material

The seeds of Mung bean var. GM-2 was procured from Agronomy farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari 396 450, Gujarat, India. The seeds were surface sterilized with 0.01 % mercuric chloride ($HgCl_2$) for 1 min followed by rinsing with autoclaved double distilled water (DDW) four times, to remove all traces of $HgCl_2$. The surface sterilized seeds were used in further experiment.

PGPR treatment

The potential PGPR isolate (*Serratia liquefaciens* isolate KM2) provided by Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari was used for seed treatment of surface sterilized Mung bean seeds. The overnight grown culture in log phase of division was used for seed treatment at the rate of 10 ml kg^{-1}. The treated seed were shed dried and then, sown in bags containing aseptic potting mixture (50 sand: 50 soil ratio). The bags were watered and nourished with hoagland solution as per need. A suitable control was kept throughout the experiment.

Physiological analysis

The root lengths, root volume, shoot length and fresh biomass analysis was done at 10 and 20 Days After Sowing (DAS). The uprooted plants were washed under clean water and then air dried before analysis. For root and shoot length was measured with a scale from below and above the collar region at root/shoot junction. The root volume was measured by dipping the roots into measuring cylinder and

calculating the rise in volume. The fresh biomass was measured using weighing balance.

Biochemical analysis

Protein content from leaves tissue was estimated by method of Lowry *et al*.[10]. The method of Diaz *et al*.[11] was used for estimation of phenol. The Chlorophyll content from leaves tissues was estimated using method of Douglas *et al.*[12.] The phytohormones *viz.*, gibberellic acid (GA_3), indole acetic acid (IAA), abscisic acid *(*ABA*)* and jasmonic acid (JA) were analyzed by method of Yuan *et al.*[13] using Ultra Fast Liquid Chromatography (UFLC; Shimadzu, Japan).

RESULTS AND DISCUSSION

The seed treatment with *Serratia liquefaciens* isolate KM2 improved Mung bean physiological parameters *viz.*, root length, root volume, shoot length and fresh biomass (Fig.1; Table 1). The *SL*-KM2 seed treatment increased root length which was reported 22.5 and 30.3 cm as compared to control in which it was observed 11.5 and 17 cm at 10 DAS and 20 DAS, respectively. The root volume was also improved by seed treatment which was 1.513 ml as compared to 0.826 ml under control condition at 10 DAS. At 20 DAS, 1.990 ml root volume was observed under *SL*-KM2 seed treatment as compared to 1.124 ml in control. The fresh biomass of Mung bean leaves at 10 DAS was 0.48 g under *SL*-KM2 seed treatment whereas 0.28 g under control condition. At 20 DAS also higher fresh biomass (1.02 g) was observed under *SL*-KM2 seed treatment.

The seed treatment with *SL*-KM2 also influenced the biochemical contents *viz.* cholorphyll, protein, and phenol in Mung bean at 10 and 20 DAS (Fig. 2). The chlorophyll A content 0.35 and 0.49 mg g^{-1} was observed under seed treatment condition as compared to 0.22 and 0.37 mg g^{-1} in control at 10 and 20 DAS, respectively. The chlorophyll B content was also improved by seed treatment which was 0.20 and 0.24 mg g^{-1} as compared to control where it was 0.18 and 0.22 mg g^{-1} at 10 and 20 DAS, respectively. The increase in both chlorophyll A and B under seed treatment showed the higher total chlorophyll content. The *SL*-KM2 seed treatment increased protein content in leaves tissue which was 1.24 and 1.63 % as compared to control where it was observed to be 0.97 and 0.96 % at 10 and 20 DAS, respectively. The phenol content in root tissue was also improved by seed treatment which was 0.21 % as compared to 0.16 % in control at 10 DAS. At 20 DAS also same trend was observed where maximum 0.23 % phenol content was reported under *SL*-KM2 seed treatment as compared to 0.17 % in control. The total phenol content in leaves tissue of Mung bean was 0.18 % under *SL*-KM2 seed treatment whereas 0.139 % under control condition at 10 DAS. The *SL*-KM2 seed treatment leads to the highest total phenol content (0.24%) as compared control (0.20 %) at 20 DAS.

The seed treatment with *SL*-KM2 influenced the phytohormones *viz.* gibberelic acid (GA_3), indole acetic acid (IAA), abscisic acid (ABA) and jasmonic acid (JA) concentration in leaves and root tissues of Mung bean (Fig. 3). The GA_3 content in root was 5.82 and 9.01 nM g^{-1} FW as compared to 3.83 and 6.28 nM g^{-1} FW in control at 10 and 20 DAS, respectively. The *SL*-KM2 seed treatment also increased GA_3 content in leaves tissue that was 34.01 and 64.56 nM g^{-1} FW as compared to control in which it was observed 30.90 and 53.23 nM g^{-1} FW at 10 and 20 DAS, respectively. The seed treatment increased IAA content in roots (32.52 nM g^{-1} FW) and leaves tissues (9.16 nM g^{-1} FW) as compared to control in which it was 20.65 and 6.41 nM g^{-1} FW, respectively at 10 DAS. At 20 DAS also, the same trend was observed. The ABA content in leaves tissue was decreased by SL-KM2 seed treatment at 10

and 20 DAS whereas; in root tissue it was found to be increased. The seed treatment showed higher ABA content 1.89 and 3.33 nM g^{-1} FW in root tissue as compared to 1.75 and 2.15 nM g^{-1} FW at 10 and 20 DAS, respectively. The JA content in root tissue was increased by seed treatment which was 3.89 nM g^{-1} FW as compared to 2.98 nM g^{-1} FW observed in control at 10 DAS. At 20 DAS also, the same trend was observed where *SL*-KM2 treatment showed higher JA content (3.77 nM g^{-1} FW). In case of leaves tissue, the JA content was found higher (5.77 nM g^{-1} FW) as compared to control (5.52 nM g^{-1} FW) at 10 DAS whereas, it was slightly decreased at 20 DAS.

In present study, seed treatment with SL-KM2 improved Mung bean physiological parameters, further it also increased biochemical content *viz.,* chlorophyll, total phenol and protein content. The increased in gibberellic acid and indole acetic content in leaves and root tissue was observed due to seed treatment whereas jasmonic acid and abscisic acid content was reduced in leaves tissue while it was slightly increased in root tissue. The mechanisms of plant growth promotion by PGPR includes associative nitrogen fixation, lowering of ethylene levels, production of siderophores and phytohormones, induction of pathogen resistance, solubilization of nutrients, promotion of mycorrhizal functioning, decreasing polluting toxicity etc[6]. Qureshi *et al.* co-inoculated phosphate solubilizing *Rhizobium phaseoli* (M_4) and *Bacillus megaterium* (B_4) having higher P solubilizing and auxin biosynthesis potential and found enhanced pod yield, straw yield, root length, dry mass and plant and grain N, P content as compared to un-inoculated control in Mung bean[7]. The CMG 860 strain capable to produce IAA and IBA significantly improved shoot and root growth in Mung bean. Its treatment improved lateral roots development where densely covered root hairs were observed, whereas very few or none developed lateral roots was found in control plants[8]. Abbas reported that application of *Bacillus licheniformis* significantly improved plant growth along with increase in carotenoids, total carbohydrates, magnesium, nitrogen and phosphorus content. The *SL*-KM2 seed treatment also influenced the production of phytohormones in Mung beans which enhanced growth and development. The application of *Bacillus licheniformis* on field bean leads to increase auxin and gibberellic acid content in shoot whereas reduced abscisic acid content[9].

Table 1. Effect of *SL*-KM2 seed treatment on physiological parameters of Mung bean at different time interval

Parameter	**Root length (Cm)**		**Root Volume (ml)**	
Interval	**10 DAS**	**20 DAS**	**10 DAS**	**20 DAS**
So	11.5	17.0	0.826	1.124
Si	22.5	30.3	1.513	1.990
Parameter	**Shoot length (Cm)**		**Fresh biomass (g)**	
Interval	**10 DAS**	**20 DAS**	**10 DAS**	**20 DAS**
So	5.0	7.5	0.28	0.704
Si	8.1	10.0	0.48	1.02

Note : So - without *SL*-KM2 seed treatment
Si - with *SL*-KM2 seed treatment

So **Si** **Root and Shoot Growth**

10 DAS

So **Si** **Root and Shoot Growth**

20 DAS

Fig. 1: Effect of *SL*-KM2 isolate on growth of Mung bean at different time interval

Note : So - without *SL*-KM2 seed treatment

Si - with *SL*-KM2 seed treatment

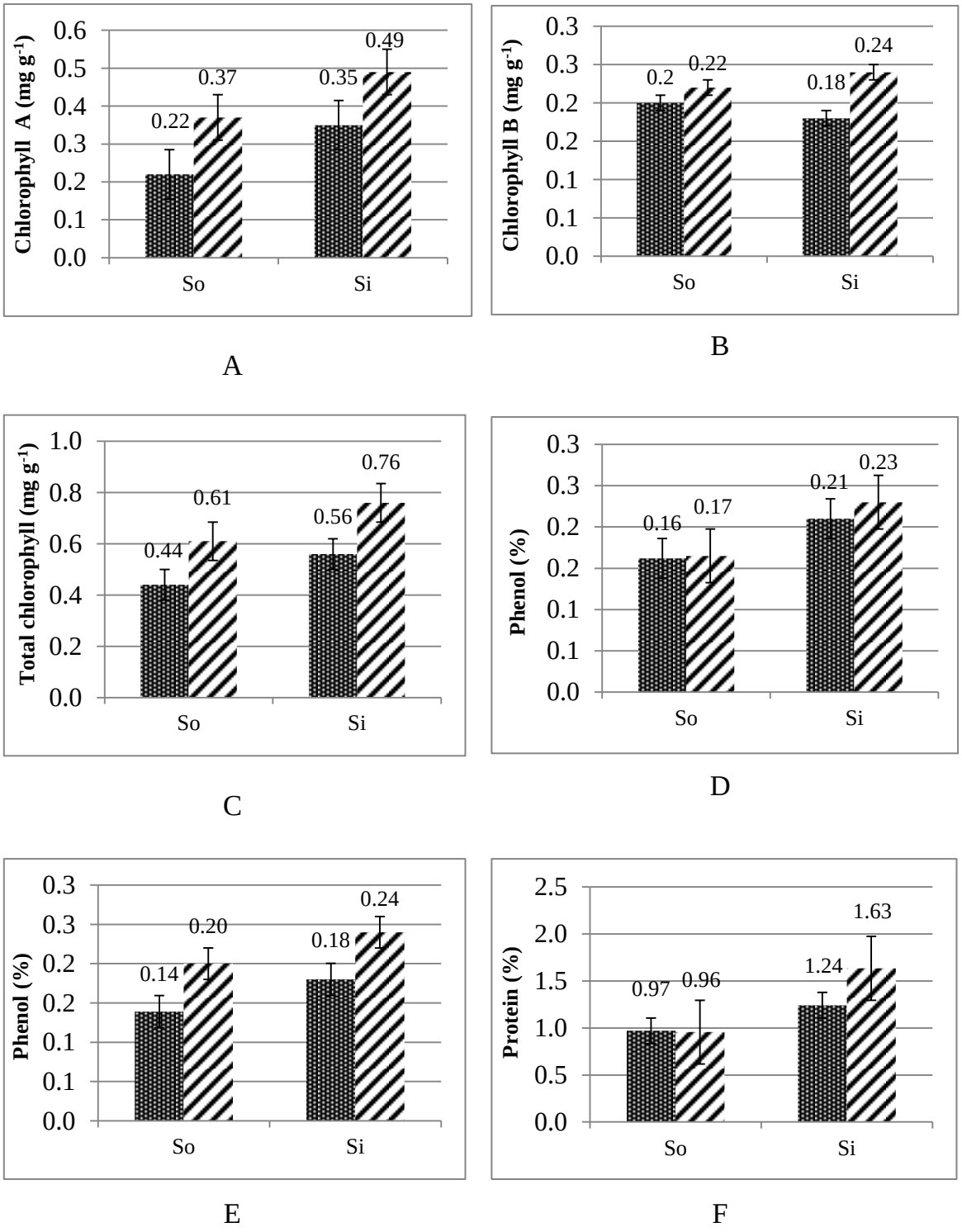

Fig. 2 : Effect of *SL*-KM2 seed treatment on biochemical content at different time intervals, A. Chlorophyll A; B. Chlorophyll B; C. Total Chlorophyll; D. Total Phenol (leaves); E. Total Phenol (roots); F. Protein Content

Note : So - without *SL*-KM2 seed treatment
Si - with *SL*-km2 seed treatment

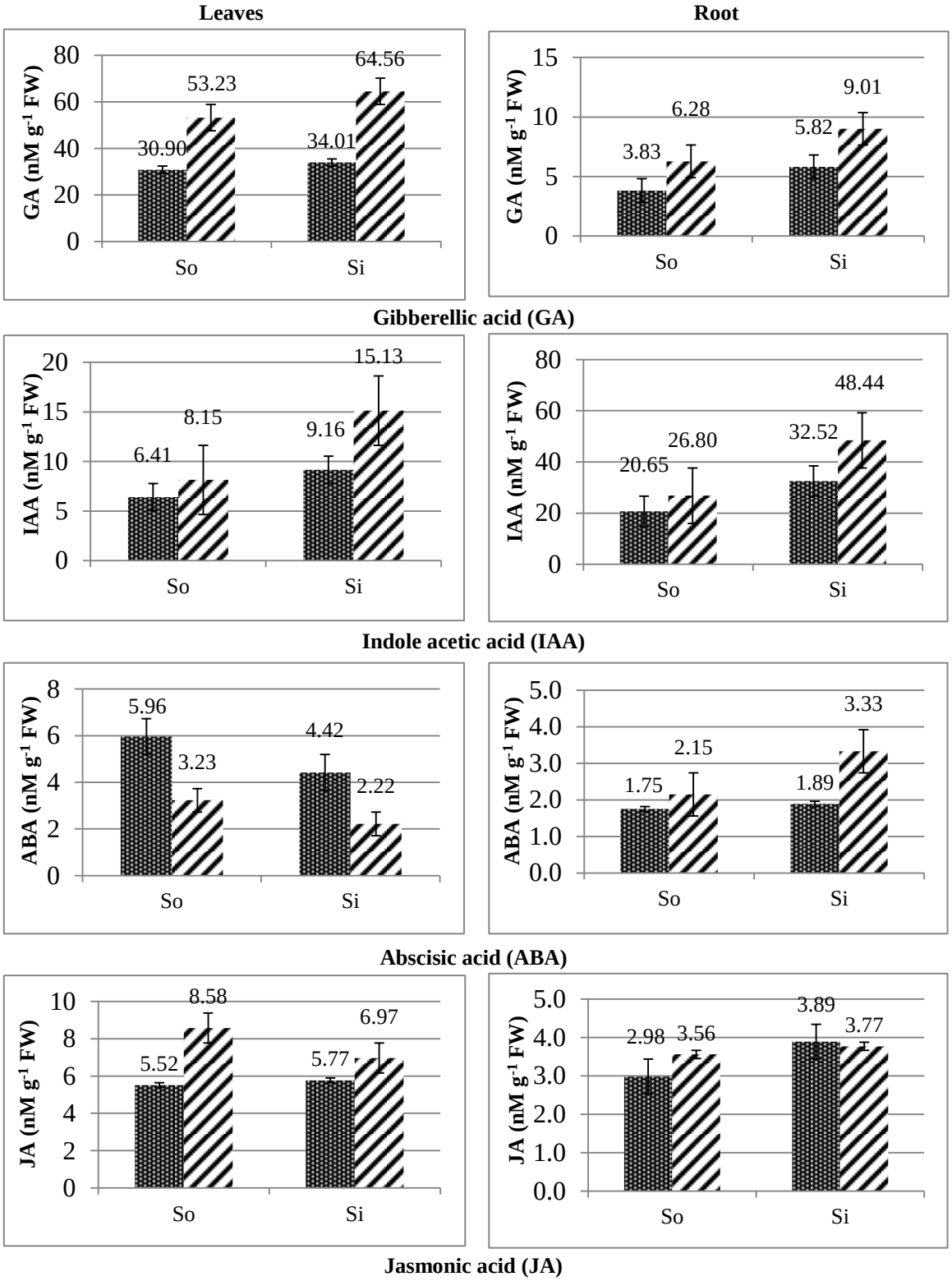

Fig. 3: Effect of *SL*-KM2 seed treatment on phytohormones content of Mung beans at different time intervals

Note : So - without *SL*-KM2 seed treatment; **Si -** with *SL*-km2 seed treatment

CONCLUSION

The *Serretia liquifecians* isolate KM2 seed treatment lead to improve the physiological parameters *viz.*, root length, root volume, shoot length and fresh biomass, further it also improved the biochemical contents *viz.*, chlorophyll, protein and total phenol of Mung bean. This treatment increased the gibberellic acid and indole acetic acid production whereas reduced abscisic acid and jasmonic acid production there by improved the plant growth and development. So, this isolate could be in future exploited for improving growth and yield of Mung bean.

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