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Full Length Article

Screening of PGPR Isolates for Plant Growth Promotion of *Rosa damascena*

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Abstract

The role of plant growth promoting rhizobacteria for growth and rooting in *Rosa damascena* Mill. cuttings was evaluated in this study. Fifteen distinctive and fast growing rhizobacterial colonies (strains) were chosen to inoculate the *Rosa* cuttings by dipping in them. Growth parameters like shoot length, new shoot fresh weight, new shoot dry weight, root length, root fresh weight and root dry weight showed highly significant results while mortality percentage of cutting was non-significant. Results of the pot experiments revealed that most of the isolates showed growth promoting activities in *Rosa* cuttings except the isolates B_5 which was proved to be growth limiting strain as it gave 20–40% less result for all parameters. The most prominent results were produced by isolated strains F_6 (*Pseudomonas fluorescens*), LSI_{19} (*Rhizobium leguminosarum*) and LC_4 (*Vibrios vulnificus*). Isolate LSI_{19} showed maximum shoot length, new shoot fresh and dry weights with an increase (over un-inoculated control) of 51.6%, 55.2% and 48.0%, respectively. Root growth was positively influenced by isolate F_6 with maximum root length and root fresh and dry weights (173.0%, 68.7% and 124.7%, respectively). All the other strains enhanced cutting growth characters up to 30–60% compared to control. Strains LSI_{19}, F_6 and LC_4 were proved to be superior strains in all growth attributes. Results on the bases of total score obtained by each strain in all growth parameters showed that strains LSI_{19}, F_6 and LC_4 got first three positions. Therefore, these three strains (LSI_{19}, F_6 and LC_4) were selected for further studies.

Keywords: Damask rose; Cutting; PGPR; Growth promotion; Environment

Introduction

The genus *Rosa* is widely distributed all over the world with approximately 200 species and 20,000 cultivar (Gudin, 2000). It is one of the most economically important genera of ornamental, medicinal and aromatic plants (Cuizhi and Robertson, 2003; Hassanein, 2010; Nadeem *et al.*, 2015). Among fragrant *Rosa* species which can produce oil, *Rosa damascena* Mill., commonly known as the Damask rose is one of the most important oil producing species (Younis *et al.*, 2008). Many aspects are involved in the higher production of flowers and quality of rose oil, among them propagation methods plays an important role. Although, different methods of propagation including the divisions of old plant, lateral sprouts with roots, seeds and micropropagation are being practiced but propagation through one year old stem cuttings is commercially adopted. The main benefits of using cuttings for propagation are the availability of genetically alike material and higher production with greater efficiency but the problem of this method is the root system, which is not always well developed (Ginova *et al.*, 2012).

Different approaches are being used like application of hormones to enhance rooting so both natural and synthetic hormones are being used in vegetative propagation of plants from stem and leaf cuttings. Different hormones especially synthetic auxins are largely used in commercial applications but their use has come under close inspection by environmental groups due to possible health hazards (Ördög, 2011). The excessive use of chemicals including fertilizers and growth hormones for a long period of time in plant production may lead to degradation of the adjacent environment. Consequently, in recent times awareness in sustainable, eco-friendly and organic cultivation practices in agriculture and horticulture has been revived (Esitken *et al.*, 2006; Dursun *et al.*, 2010). As a result, plant growth promoting rhizobacteria (PGPR) with their growth promoting ability are spotlighted and are being applied widely to agricultural crops as this technology is quite suitable to increase plant growth and decrease the use of chemical inputs. Several mechanisms have also been reported by which PGPR can directly stimulate the growth of plants including (1) production of phytohormones like IAA, Gibberellin, Kinetin and Cytokinin (Noel *et al.*, 1996;

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Antoun *et al.*, 1998; Verma *et al.*, 2001; Dey *et al.*, 2004) and (2) enzymes generation that can alter growth and development of plants (Yang and Hoffman, 1984; Sharma *et al.*, 2011). The production of growth promoting substances like gibberellins, cytokinins and auxins by inoculated bacteria have positive impact on plant growth and yield. These substances accelerate the growth of root system and may also alter the balance of endogenous plant phytohormones. Another beneficial aspect of PGPR bacteria is promotion of rooting capacity and control over the soil born pathogenic communities of microbes by producing B-group vitamins and antibiotic metabolites (Dobbelaere *et al.*, 1999; Revillas *et al.*, 2000; Mostafa and Abo-Baker, 2010).

The positive impact of plant growth promoting rhizobacteria (PGPR) has been studied in annual crops like wheat, soybeans, lettuce, beans, corn and barley in several ways, but limited studies are present on woody plant species. Few studies have been conducted on roses where bacteria were isolated from the rhizoplane and the rhizosphere of *R. indica* and used for growth promotion of *Rosa* and apple plants (Caesar and Burr, 1987; Chanway and Holl, 1993; Barazani and Friedman, 1999). This opened up new possibilities to use PGPR for propagation of *R. damascena*, and application of beneficial bacteria may stimulate plant growth. Therefore, it is necessary to choose superior, naturally occurring rhizospheric bacteria which may enable us to achieve maximum plant growth. This introduction of environment friendly technology may lead to sustainable rose production. Keep in view the benefits of plant growth promoting rhizobacteria (PGPR), present study has been planned to screen plant growth promoting rhizobacteria (PGPR) for growth promotion of *R. damascena* cuttings.

Materials and Methods

The proposed experiment was carried out at the Rose Project, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (31°30'N, 73°10'E and altitude 213 m above sea). Fifteen pre-isolated and prescreened PGPR strains were acquired from Soil Microbiology Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. The fifteen strain used for screening were B_5, R_6, J_1, LC_3, F_6, LC_4, LSI_{29}, LSI_{30}, JH_4, LSI_{19}, CRI_{31}, CRI_{34}, A_{18}, S_6 and PsJN. These strains were identified as *Burkholderia phytofirmans* (PsJN), *Mesorhizobium ciceri* (CRI_{31}, CRI_{34} and JH_4), *Pseudomonas fluorescens* (F_6 and B_5), *Rhizobium leguminosarum* (LSI_{19}, LSI_{29} and LSI_{30}), *Rhizobium phaseoli* (A_{18} and S_6), *Vibrios diazotrophicus* (J_1 and R_6) and *Vibrios vulnificus* (LC_3 and LC_4).

Screening experiment was conducted using sterilized plastic pots. First of all, a nutrient broth was prepared in conical flask for inoculation of bacterial strains. Each flask containing 100 mL of broth was sterilized and was inoculated with all selected strains of rhizobacteria. Selected rhizobacterial strains in the conical flasks were cultured in a shaking incubator with 100 rpm (Firstek Scientific, Tokyo, Japan), at 28±1°C for 48–72 h. Damask rose stem cuttings were taken during the first week of December from Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture Faisalabad. In order to inoculate the cuttings, basal end of each cutting was dipped in 2 mL of bacterial inoculum for 45 min (Karakurt *et al.*, 2009). For this purpose, bundle of 20 cutting were made and the cuttings were dipped in 40 mL of bacterial inoculum. The inoculated cuttings were planted in plastic pots filled with sterilized sand. This experiment was laid out in completely randomized design (CRD) with four replications. There were 16 treatments containing uninoculated control and 15 strains of PGPR. For control treatment cuttings were dipped in sterilized nutrient broth. Treatments comprised of control (Unioculated), B_5, R_6, J_1, LC_3, F_6, LC_4, LSI_{29}, LSI_{30}, JH_4, LSI_{19}, CRI_{31}, CRI_{34}, A_{18}, S_6 and PsJN. Water was applied by maintaining initial weight of pot with soil + water and data were collected regularly till 6 months for mortality percentage (%), length of new shoot (cm), fresh weight of shoot (g), dry weight of shoot (g), root length (cm), fresh weight of root (g) and dry weight of root (g).

Best microbial strains were ranked by percent increase or decrease from control treatment for all growth attributes. For this purpose, scoring was done from 0 to 10 and scores for each growth attribute were compared individually for each strain. Each strain was given a scores on the bases of variation in percentage from control, therefore, score were assigned as 0=less or equal to 0%, 1= 1–10%, 2= 11–20%, 3= 21–30%, 4= 31–40%, 5= 41–50%, 6= 50–60%, 7= 61–70%, 8= 71–80%, 9= 81–90% and 10= 91–100%. Finally, all the strains were ranked on the bases of total score obtained by each strain in all growth parameters. The strains that got three top positions were subjected for further studies.

Statistical Analysis

Experimental data collected for parameters were analyzed by using the Fisher's analysis of variance technique and Least Significant Difference (LSD) test at 5% probability to compare the treatment means (Steel *et al.*, 1997).

Results

In this experiment, *R. damascena* cuttings were inoculated with the bacterial strains. Response of different growth parameters in relation to different PGPR strains were observed and collected data was analyzed statistically using analysis of variance technique (Table 1) and results were subjected to LSD test at 5% probability for the comparison of the treatment means. The application of PGPR inoculum to *R. damascena* cuttings showed non-significant results for mortality percentage while highly significant results for

shoot length, new shoot fresh weight, new shoot dry weight, root length, root fresh weight and root dry weight were observed (Table 1). The association of cuttings' mortality with the application of PGPR inoculum was statistically unaffected, while maximum but not desirable mortality percentage was observed in PGPR strain (B_5). The cuttings treated with LC_4 and LSI_{19} showed no mortality, these two strains proved to be much suitable strains for survival of cuttings as compared to other treatments (Fig. 1). The results regarding maximum shoot length with 51.6% and 48.9% increase in shoot length over un-inoculated control were produced by the isolates LSI_{19} and F_6, respectively. The cuttings treated with PGPR strain B_5 showed lowest results in shoot length with 19.7% less shoot length than un-inoculated control (Fig. 2). Maximum shoot fresh weight with 55.2% increase over un-inoculated control was shown in LSI_{19} followed by 39.1 and 32.3% increase was observed with the isolates F_6 and LC_4 respectively (Fig. 3). The cuttings treated with PGPR strain B_5 showed lowest results with 31.8% less shoot fresh weight than un-inoculated control. Similarly, the results regarding effect of PGPR treatments on new shoot dry weight of *R. damascena* showed maximum shoot dry weight with 48.0% increase over un-inoculated control in LSI_{19} followed by 35.7% increase was shown by the isolates F_6 (Fig. 4). The cuttings treated with PGPR strain B_5 showed lowest results with 24.1% less shoot fresh weight than un-inoculated control.

The results regarding effect of PGPR treatments on root length of *R. damascena* are given in Fig. 5 as treatment means. Maximum root length with 173.0% increase over un-inoculated control was shown in F_6 followed by 118.4% increase over un-inoculated control was shown by isolate LSI_{19}. Root fresh weight with an increase of 68.7% over un-inoculated control was shown in F_6 followed by 63.1% increase over un-inoculated control by the isolate LSI_{19}. In the same way, isolate F_6 showed maximum root dry weight with 124.7% increase over un-inoculated control followed by isolates LSI_{19} and LC_4 where 79.8% and 54.2% increase over un-inoculated control was observed. The cuttings treated with PGPR strain B_5 showed lowest results root fresh and dry weight with 45.0% and 37.5% less results, respectively. On the other hand, all other strain behaved almost in equal manner in all growth parameters.

Finally all microbial strains were ranked by percent increase or decrease from control treatment for all growth attributes (Table 2). Results on the bases of total score obtained by each strain in all growth parameters showed that strains LSI_{19}, F_6 and LC_4 got top three positions. Therefore, these strains were selected are recommended for further experiments.

Discussion

Results of screening trials showed that most of the plant growth promoting rhizobacteria used in this study exhibited growth increasing behaviour in damask rose. The

Table 1: Mean squares of different growth characteristics of *Rosa damascena* cuttings

Source of Variation	Treatments	Error
D.F	14	48
Mortality (%)	302.94^{NS}	444.14
New shoot length (cm)	58.42**	2.97
Shoot Fresh Weight (g)	14.78**	0.4
Shoot Dry Weight (g)	2.34**	0.05
Root Length (cm)	50.12**	0.77
Root Fresh Weight (g)	2.51**	0.043
Root Dry Weight (g)	0.104**	0.002

*and ** denotes differences significant (P<0.05) and highly significant (P<0.01) respectively and ^{NS} denotes non-significant values

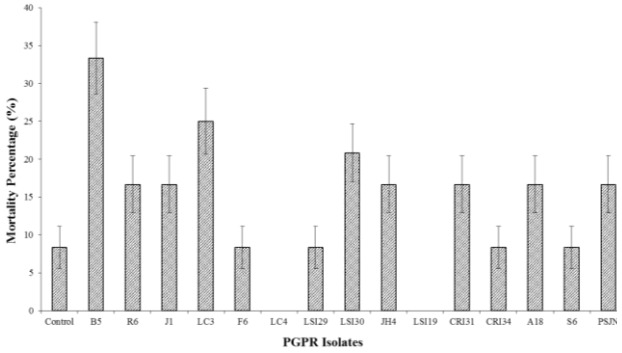

Fig. 1: Efficacy of various PGPR strains on Mortality Percentage of cuttings

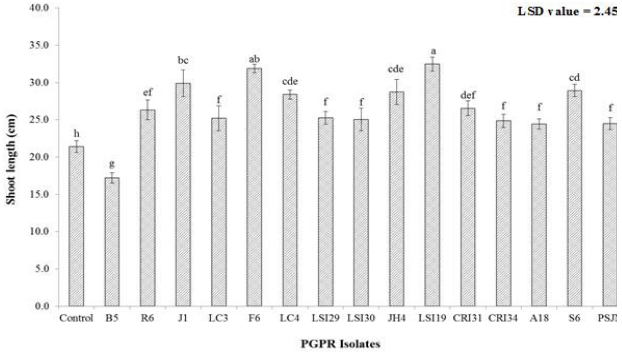

Fig. 2: Efficacy of various PGPR strains on shoot length of cuttings

modification in growth and development of damask rose by these PGPR isolates may be because of different mechanisms of actions like production of plant hormones and siderophore, solubilization of insoluble Phosphate and increased nutrient uptake (Pena and Reyes, 2007; Nehra *et al.*, 2014; Spaepen, 2015). This may imply that the ability of PGPR isolates with different mechanisms of actions is responsible for inducing the growth stimulating response in the inoculated cuttings of damask rose. In this experiment, application of PGPR strains significantly affected the cuttings growth only the results regarding cuttings survival percentage were non-significant. Similar, results were

Table 2: Scoring of various PGPR strains on the basis of growth percentage with respect to control

Strains	Mortality percentage (%)	Length of new shoot (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Root Length (cm)	Total
LSI19	10	6	6	5	7	6	10	50
F6	10	5	4	4	7	8	10	48
LC4	10	4	4	4	6	5	9	42
CRI34	10	2	0	0	5	4	7	28
LSI29	10	2	1	1	4	3	6	27
S6	10	4	1	1	3	2	4	25
JH4	0	4	2	2	3	2	5	18
LSI30	0	2	1	1	4	3	6	17
CRI31	0	3	0	0	4	3	6	16
J1	0	4	3	3	0	1	0	11
A18	0	2	0	0	3	2	4	11
LC3	0	2	1	1	2	1	3	10
PSJN	0	2	0	0	2	1	3	8
R6	0	3	0	0	0	0	0	3
B5	0	0	0	0	0	0	0	0

reported by Karakurt *et al.* (2009) where bacterial treatments provided the equal rooting rate in single treatments. Propagation of damask rose from cuttings is easiest and mass production method but it totally depends on the ability of cuttings to produce new roots and shoots. In matter of fact, all cuttings taken from a plant do not survive as most of them are unable to produce roots due to internal and external factors like imbalance production of hormones, induction of diseases, humidity, temperature and improper substrates. Most of the cuttings pronounced to survival problem might be due to nutritional status of the stock cuttings. It was also reported by Smalley *et al.* (1991) that a minimal level of carbohydrate was needed for stock cuttings to survival, to run physiological activity and to emerge roots (Saifuddin *et al.*, 2013). It is suggested that survival rate and root initiation can be increased by applying endogenous rooting hormones and nutrients before plantation (Soundy *et al.*, 2008; Mori *et al.*, 2011; Ahmad *et al.*, 2012). Therefore, different types of synthetic hormones are being used, among them auxins play a vital role in influencing the root formation and survival rate of stem cuttings (Raju and Prasad, 2010; Severino *et al.*, 2011; Zafar-ul-Hye *et al.*, 2014). But in order to avoid hazards of hormones, the inoculated bacteria which produces growth promoting substances like gibberellins, cytokinins and auxins are alternative to synthetic rooting hormones and have positive impact on plant growth and yield. These substances produced by microbes accelerate the growth of root system and may also alter the balance of endogenous plant phytohormones (Martínez-Viveros *et al.*, 2010).

The results of the present experiment indicated that the efficiency of the PGPR isolates differs among the species when applied to a similar host plants. The difference in affectivity amongst the PGPR isolates to a similar host plants might be due to variation in their characteristics particularly their root colonization ability with host plants, which is evident from the results of characterization of the selected PGPR isolates of damask rose. Therefore, rooting characteristics like fresh and dry

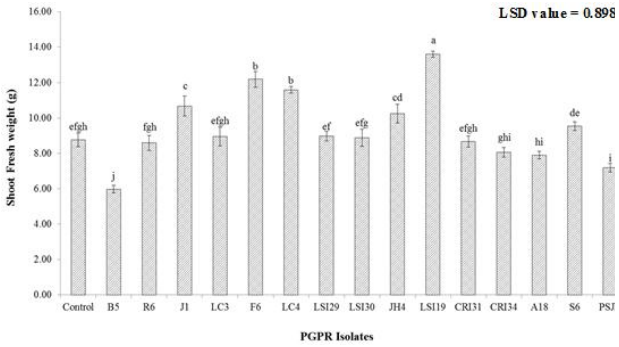

Fig. 3: Efficacy of various PGPR strains on new shoot fresh weight of cuttings

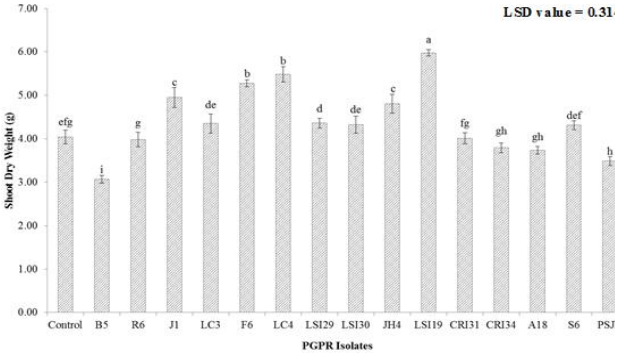

Fig. 4: Efficacy of various PGPR strains on new shoot dry weight of cuttings

weight of roots and root length differ a lot among all applied PGPR isolates. Isolate F_6 (*Pseudomonas fluorescens*) gave the highest results for rooting characteristics might be due to increase in the amount of auxin near the root zone which resulted in higher root length and biomass production. The role of auxins especially IAA for healthier and abundant rooting in many plants is previously stated (Hartman and Kester, 1972) and

similar results with the application *P. fluorescens* to black pepper (*Piper nigrum*) rooted cuttings were reported by Thankamani *et al.* (2005). Plant growth promoting characteristics of *Pseudomonas* rhizobacteria promote root and shoot growth that's why plants treated with *Pantoea*, *Pseudomonas* sp. significant increase in plant growth parameters like shoot and root weight and total biomass on fresh weight and dry weight basis in Coleus (Damam *et al.*, 2014). It has often been derived that rhizobacterially produced auxins are responsible for growth promotion but indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway (Kende, 1993). Another evidence related to better rooting was control of pathogens which limits the root growth by decaying process, it has been reported that many *Pseudomonas* species have effective ability to control pathogenic fungi, mainly Fusarium (Paulitz and Belanger, 2001; Rosas *et al.*, 2001). Similar results were reported by Felker *et al.* (2005) root pathogens which are major problem in rooting Prosopis cuttings were overcome by using *Pseudomonas aurantiaca*.

In the rhizhosphere, production of ethylene results in inhibition of root elongation. Apart from this the plants treated with rhizobacteria, the ethylene production is subdued by aminocyclo propane carboxylic acid deaminase which produces ammonia instead of ethylene so this leads to rapid elongation of roots (Kloepper, 2003). Isolate LSI_{19} (*Rhizobium leguminosarum*) also showed better results than other isolates for rooting characteristics and was ranked second after F_6, these results are in line with the findings of Noel *et al.* (1996). They carried out a study where they used parent and mutant strains of *Rhizobium leguminosarum* to inoculate the seeds of canola and lettuce where they noticed significant improvement in growth of seedlings root by inoculation with some strains. They concluded that the enhanced root growth come out perhaps as results of IAA and cytokinins production.

Results regarding maximum shoot length showed that the treatments comprising strain LSI_{19} (*Rhizobium leguminosarum*) have significantly superior effect, it is mainly due to ability *Rhizobium* strain LSI_{19} to fix atmospheric nitrogen and make it available to plant roots. Increase in the shoots length due to higher dose of nitrogen and phosphorus application had also been reported by Tajuddin *et al.* (1986) in *R. damascena*. On the other hand, the noticeable increase in shoot length in the presence of PGPR was recorded due to the improved IAA availability. This improved availability of IAA induced cell division and cell elongation which ultimately improved plant height (Mohite, 2013; Ul Hassan and Bano, 2015). Similarly, an increase in carnation growth in early stage of plant development as compared to un-inoculated plants was observed when plants were inoculated with *Rhizobium* starins (Menéndez *et al.*, 2016). Fresh and dry weights of new shoot were also greatly influenced by all inoculum of PGPR strains especially LSI_{19} (*Rhizobium leguminosarum*).

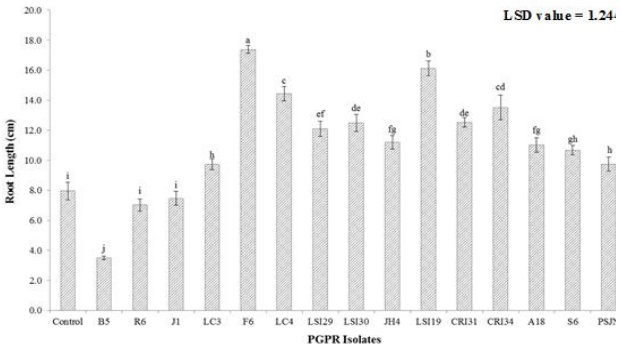

Fig. 5a: Efficacy of various PGPR strains on root length of cuttings

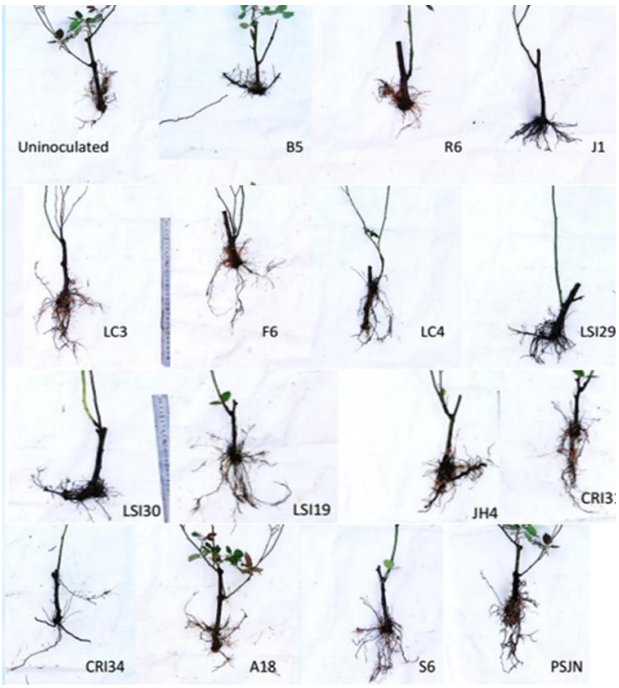

Fig. 5b: Pictorial view of root length of cuttings by PGPR strains application

The increase in weights was due to absorption of more nutrients and their utilization. The core reason of ideal rate of nutrients absorption by the plants was attributed due to biological fixation of nitrogen (BNF) and solubilisation of phosphorus in root zone of plants (Qasim *et al.*, 2014). In the same way, increase in fresh and dry weight of plants was also reported due to the role of PGPR in improving organic matter in the soil along with elevated nutrients availability (Sasirekha *et al.*, 2012). The application of *Rhizobium* sp. PEPV12 showed significant increase in shoot length of spinach with respect to un-inoculated plants (Jiménez-Gómez *et al.*, 2016). Several other studies also reported that plants showed superior morphological growth due to soil inoculation to increase supply of nitrogen, through BNF,

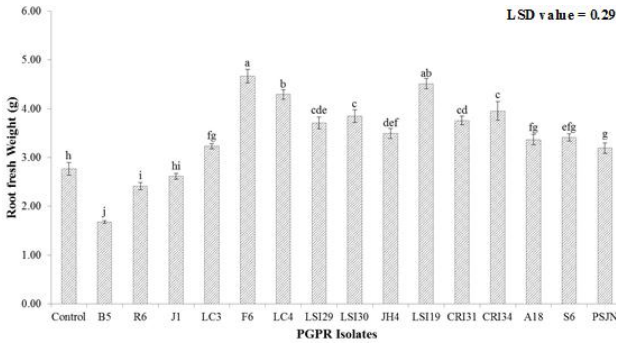

Fig. 6: Efficacy of various PGPR strains on root fresh weight of cuttings

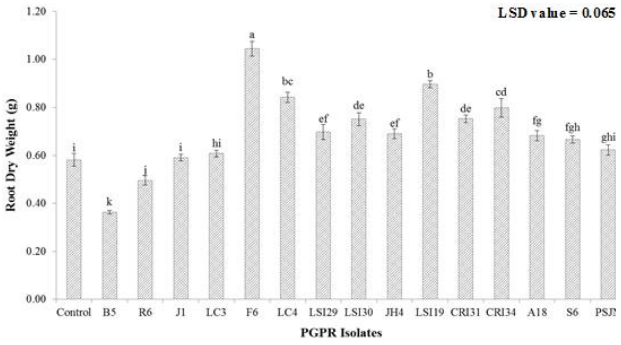

Fig. 7: Efficacy of various PGPR strains on root dry weight of cuttings

and solubility of phosphorus (Togay *et al.*, 2008; Ahmed *et al.*, 2010; Khan *et al.*, 2014).

In general, most of the isolates showed growth promoting effects in damask rose in all aspects which may be due to previously stated growth promoting mechanisms, but growth limiting effect by PGPR strain B_5 was observed in all growth parameters. This growth limiting behaviour may be observed due to excessive production of materials (IAA and similar substances) that are beneficial for plant growth at minor quantity (Antoun *et al.*, 1998). In the same way, Alstrom and Burn (1989) have also reported for growth limiting effects on the growth of plants which is attributable due to the production of HCN at higher concentration. Moreover, reduction of plant growth had been reported with the application of rhizobial inoculation to non-legumes, which might be associated to the production of growth inhibitors by the rhizobial strains (El-Tarabily *et al.*, 2006). Despite the promising PGPR characters strain B_5 showed negative results but other strains like F_6 (*Pseudomonas fluorescens*), LSI_{19} (*Rhizobium leguminosarum*) and LC_4 (*Vibrios vulnificus*) showed effective root colonization and encourage growth and development of plant in early stages. Further evaluation should be executed in order to clarify the reason of negative impact of strain B_5 on plant growth.

Conclusion

On the basis of these results of the study, it is suggested that the way followed for the selection of fast growing isolates and assessment of their growth promoting potential on cuttings of damask rose could be a suitable method for screening the of effective and active isolates. Ultimately, strains F_6 (*Pseudomonas fluorescens*), LSI_{19} (*Rhizobium leguminosarum*) and LC_4 (*Vibrios vulnificus*) are found to be the most effective strains among all tested strains so these strains are recommended for further studies to assess the potential of strains for yield and oil contents.

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