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AN ASSESSMENT OF PLANT GROWTH PROMOTING RHIZOBACTERIAL STRAINS FOR GROWTH OF *SPINACIA OLERACEA* UNDER *IN-VIVO* CONDITIONS

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are beneficial bacterial strains found in rhizosphere that colonize plant roots and enhances plant growth by wide mode of mechanisms. The usage of PGPR is increasing day by day in the field of agriculture and it offers a sensible way to replace chemical fertilizers, pesticides and other supplements. This preliminary study was carried-out to evaluate nine rhizobacterial strains associated with *Spinacia oleracea* for different plant growth parameters i.e. root length, shoot length, number of leaves and area of leaves, under *in-vivo* conditions. The tested rhizobacterial strains were isolated in a previous study from soil that had been cultivated with *Spinacia oleracea* for at least three consecutive seasons. This study is further extension of our work which involved the *in-vitro* analysis of the rhizobacterial strains for different plant growth promoting activities. All the tested rhizobacterial strains showed positive performance in terms of improvement in plant growth parameters when compared to that of control . Strains R13, R40, R50, and R53 showed best plant growth parameters under *in-vivo* conditions. Furthermore upon completion of one month, the shoot length of plants inoculated with isolate R13, R40, R50, R53 were found to be 32cm, 26.5cm, 34 cm, 38.6cm respectively, compared to control i.e. 21 cm, whereas root length of plants inoculated with same isolates were found to be 8cm, 15cm, 12.5cm, 8cm respectively, compared to control i.e. 9.5 cm. Number of leaves per plant, which were inoculated with these respective strains were found to be 8 units, 6 units, 8 units, and 7 units respectively, compared to control i.e. 6 units, and leaf area of these plants which were inoculated with same isolates were found to be $20cm^2$, $18.15cm^2$, $20.07cm^2$, and $13cm^2$ respectively, compared to control i.e. 19.68 cm^2. These results suggest that isolate R13, R40, R50, R53 may be tested further for more plant growth promoting traits eventually developing it as potential soil inoculants in order to enhance the growth of *Spinacia oleracea*.

KEYWORDS: Rhizobacteria; Rhizosphere; Plant growth promotion

INTRODUCTION

Over the last decades, huge increase in the crop yield has been reported which proved beneficial for the starving population. However, this benefit turned into a nightmare for our environment as these increase in crop-yield is at the cost of our environment, in other words continued usage of chemical fertilizers and pesticides led to the many serious environmental problems like pollution, bioaccumulation, alteration of soil texture, eutrophication, deleterious effects on soil and its quality, soil-erosion, reduction in fertility of soil, and reduction in the populations of naturally occurring beneficial microbial strains (which helps in growth promotion of plants) and many more (Kuhajek et al., 2003). Now, this problem is taking new shape as with ever-increasing population, when large areas of soil are used for housing, areas which are available for farmlands are reducing. Therefore, we have to look for an alternative source which can do both things for us i.e. firstly, it should be able to feed ever-increasing world population along with this, it should not harm our surrounding environment in any form. Investigations revealed that PGPR (Plant Growth Promoting Rhizobacteria) is capable to perform both things for us in a environment-friendly manner. PGPR helps in growth promotion of plant through various activities like fixation of nitrogen (Keneni et al., 2010) by increasing the availability of nutrients in the rhizospheric region of soil (Sessitsch et al., 2002) by enhancing other beneficial symbiosis of the host plant (Vikram et al., 2008) by combining mode of actions (Wipat et al., 1999) and by increasing root surface area (Antoun and Kloepper, 2001). PGPR or simply rhizobacteria are found to play an important role in increasing crop-yield and maintaining soil-health, through various activities which eventually helps in growth promotion of plant (Kloepper and Schroth, 1978). Plant growth promoting rhizobacteria

(PGPR) accounts for about 2-5% of entire rhizobacterial strains involved in plant growth promotion (Antoun and Kloepper, 2001). Some of these activities are mineral solubilisation , nutrient uptake and cycling, induction of systemic resistance in host plants, secretion of phytohormones , along with this suppression of phytopathogens and much more activities like this which eventually found beneficial for growth promotion of plants (Kloepper et al , 2004 ; Haas and Defago, 2005). Investigations revealed that soil is being utilized as home by variety of microbial strains like bacteria, fungus, algae, and protozoa, however, amongst them bacteria are most abundant (Glick and Thompson; 1993). PGPR includes variety of bacterial genera such as Pseudomonas, Bacillus, Acetobacter, Arthrobacter, Rhizobia, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas and Micrococcus etc. It was reported that three types of interactions are there between the host plant and the growing rhizobacterial strain, which is according to the Whipps (2001) is positive interaction, negative interaction and neutral interaction. In case of neutral interaction, there is no visible effect which is identified in the growth and overall physiology of the host plant (Beattie, 2006). In that interaction when phytopathogenic rhizobacteria produces phytotoxic compound like hydrogen cyanide or ethylene which imposes negative effects on the growth and development of host plant then such kind of interactions are termed as negative interactions. However, rhizobacteria is also being associated with the activities like phosphate solubilisation, secretion of phytohormones like indole-acetic acid, gibberellin etc. along with the activities like antibiotic production which helps in growth promotion of the plant, therefore such kind of interactions between the host plant and the rhizobacterial strain constitutes, the positive interaction. Also according to their degree of association with root cells of plant PGPR can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martinez–Viveros et al, 2010). iPGPRs resides inside the specialized nodular structures of root cells while ePGPRs may present in either rhizosphere or on the rhizoplane or in the spaces between the cells of the root cortex. The bacterial strains of Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Caulobacter etc. belongs to ePGPR(Gray and Smith 2005), Whereas endophytes and Frankia species both which can symbiotically fix atmospheric nitrogen with the higher plants (Verma et al. 2010) are included as a iPGPRs. PGPRs are usually found in rhizosphere, and rhizosphere is the portion of soil which surrounds plant root (Walker et al, 2003). This portion is approximately 1mm wider in diameter from the surface of the root. However, rhizosphere is a portion of soil where intense biological and chemical activities were carried out (Curl and Truelove; 1986). This is usually done because of secretions released by plant root and resident microbes of rhizosphere. And the soil which is not associated part of rhizosphere is called bulk soil. And in this portion of the soil, microbial & plant root activities were comparatively low when compared to that of rhizosphere. Plant roots has been found to secrete many complex compounds into the rhizosphere (Walker et al, 2003) which were found to serve different functions. Therefore, on considering all these we can suggest that using PGPR as biofertilizer would not be a bad choice as it is offering us economical and environment-friendly way of farming.

MATERIALS AND METHODS

Rhizobacterial strains : These rhizobacterial strains were isolated in a previous study from soil that had been cultivated with *Spinacia oleracea* for at least three consecutive seasons. This study is further extension of our work which involved the *in-vitro* analysis of the rhizobacterial strains for different plant growth promoting activities.

Screening for *in-vivo* plant growth promotion: This study was carried out during the autumn-winter 2014. Eight days old germinated seedlings of *Spinacia oleracea* were separately sown in a medium sized pot which could accommodate around 200-300 g soil, followed by inoculation with respective rhizobacterial strains. The inoculated plantlets were continuously monitored and maintained. These plantlets were further analysed for different plant growth promoting activities i.e. root and shoot length of plantlets, area of leaves and the number of leaves. After one month of regular maintenance and observation, these plantlets were completely uprooted for comparison and for noting down the measurements of different parameters which were measured on the first day of the start day of experiment. On the first day in nine pots we introduced rhizobacterial isolates i.e. R3, R6, R13, R20, R24, R35, R40, R50 and R53 respectively, and no strains were added in the control. On each pot we introduced 10 ml overnight activated cultured solution of respective rhizobacterial strains. Also, at first day we noted down the several parameters. These were number of leaves on each plantlet, length and breadth of the largest leaf of respective plantlets, area of leaf, along with this we measured shoot and root length of each plantlet. Furthermore, upon completion of one month we again noted down the readings for these growth parameters. In this experiment we individually marked each and every pot with unique serial number to remove any kind of confusion for smooth-conduction of this experiment.

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RESULTS AND DISCUSSION

In this preliminary study it was found that all the germinated seedlings which were inoculated with respective rhizobacterial strains, showed positive performance in terms of improvement in plant growth parameters than that of control . It was also found that isolates i.e. R13, R40, R50 and R53, showed best growth when compared to rest of the isolates and to the control . Furthermore, upon completion of one month the shoot length of plantlets inoculated with isolate R13, R40, R50, R53 were found to be 32 cm, 26.5 cm, 34 cm, 38.6 cm respectively, against control which showed shoot length of 21 cm. Also, root length of the plantlets inoculated with respective isolates were found to be 8 cm, 15 cm, 12.5 cm, 8 cm respectively, against control which showed the root length of 9.5 cm. The number of leaves per plant which were inoculated with these respective isolates were found to be 8 units, 6 units, 8 units, and 7 units respectively, against control in which number of leaves were found to be 6 units, and finally the leaf area of these plantlets, which were inoculated with these respective isolates were found to be 20 cm^2, 18.15 cm^2, 20.07 cm^2, and 13 cm^2 respectively, against control which showed leaf area of 19.68 cm^2. Therefore it may be concluded that germinated seedlings which were inoculated with rhizobacterial isolates i.e. R13, R40, R50 and R53 respectively, were found to show best plant growth parameters under *in-vivo* conditions.

Plant growth promoting rhizobacteria (PGPR) or simply rhizobacteria constitutes important group of bacterial community which were found to enhance plant growth promotion by colonization of root (Kloepper, 1991) and also through activities like mineral solubilisation, nutrient uptake and cycling, induction of systemic resistance in host plants, secretion of phytohormones , along with this suppression of phytopathogens and much more activities like this which eventually found beneficial for the growth promotion in plants (Kloepper et al , 2004 ; Haas and Defago, 2005). This rhizobacteria are mainly rhizospheric microorganisms, which were found in the rhizosphere of the soil, rhizosphere is described as a narrow zone of soil which is found directly surrounding the root system (Walker et al, 2003). Here, in this region (rhizosphere) plant root are found to perform various activities like synthesis, accumulation and secretion of various compounds (Walker et al, 2003). PGPR are found to secrete many compounds like siderophores (Iron-chelating compound) (Sahu et al., 2011) hydrogen cyanide (Phytotoxic compound) (Sahu et al., 2011) natural auxin (Indole-acetic acid) (Phytohormone) (Frankenberg et al., 1995) antibiotics (Phytopathogen suppressor) (Berg, 2009) and also found to solubilise mineral (Phosphate) [Rodriguez et al., 1999] which increases availability of nutrients to host plants, along with this suppression of phytopathogens by secretion of ammonia (Berg, 2009) and most important rhizobacteria is also associated with the fixation of atmospheric nitrogen (Tilak et al., 2005) to the forms which can easily be up taken by the plants and hence enhances plant growth promotion and many more compounds like this. Plant growth promoting rhizobacteria (PGPR) accounts for about 2-5% of the total rhizobacterial strains which were found involved in the plant growth promotion (Antoun and Kloepper, 2001). As soil serves as home for variety of microbial strains like bacteria, fungus, algae, and protozoa. However, bacteria are found to be most abundant amongst them (Glick and Thompson; 1993) (Glick et al., 1993). PGPR includes variety of bacterial genera such as Pseudomonas, Bacillus, Acetobacter, Arthrobacter, Rhizobia, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas and Micrococcus etc. Therefore on considering above parameters which were involved in the growth promotion of the plant, we can suggest that rhizobacteria can help in increasing crop-yield, also that a in environmental-friendly way, without any need of further usage of chemical fertilizers. These rhizobacterial strains may help in maintaining good soil-ecosystem and also it may help in increasing crop-yield that is also in a environment friendly manner.

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Figure 1 : Comparison of growth parameters of Spinacia oleracea on the first day and after one month of treatment with rhizobacterial strains.

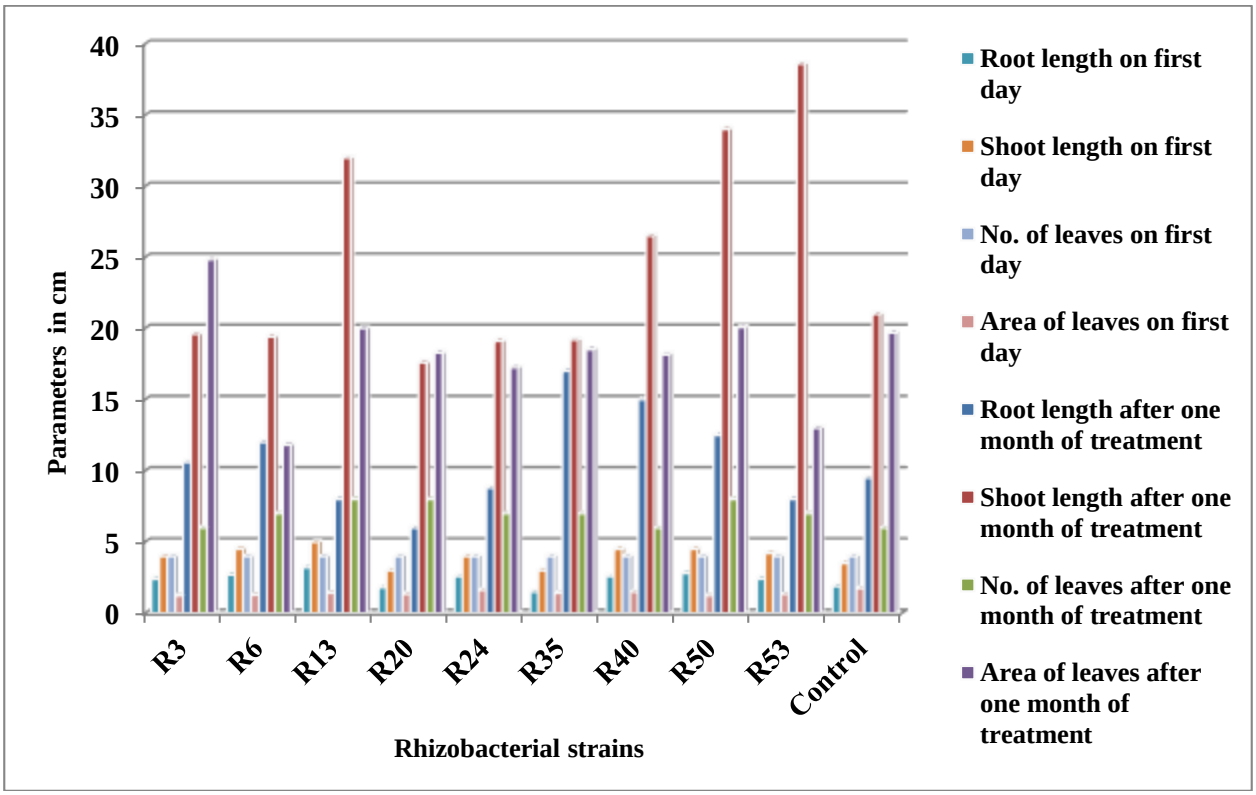

Table 1: Growth parameters on first day

Rhizobacterial strain	Pot no.	Root length	Shoot length	No. of leaves per plant	Leaf Area (lxbx0.625)
R3	1	2.4 cm	4 cm	4	1.25 cm^2
R6	2	2.7 cm	4.5 cm	4	1.27 cm^2
R13	3	3.2 cm	5 cm	4	1.42 cm^2
R20	4	1.8 cm	3 cm	4	1.35 cm^2
R24	5	2.6 cm	4 cm	4	1.62 cm^2
R35	6	1.5 cm	3 cm	4	1.42 cm^2
R40	7	2.6 cm	4.5 cm	4	1.50 cm^2
R50	8	2.8 cm	4.5 cm	4	1.23 cm^2
R53	9	2.4 cm	4.2 cm	4	1.35 cm^2
Control	10	1.9 cm	3.5 cm	4	1.75 cm^2

Table 2: Growth parameters after one month of treatment with tested rhizobacterial strains

Rhizobacterial strain	Pot no.	Root length	Shoot length	No. of leaves per plant	Leaf Area (lxbx0.625)
R3	**1**	**10.6 cm**	**19.6 cm**	**6**	**24.84 cm^2**
R6	**2**	**12 cm**	**19.4 cm**	**7**	**11.81 cm^2**
R13	**3**	**8 cm**	**32 cm**	**8**	**20 cm^2**
R20	**4**	**6 cm**	**17.6 cm**	**8**	**18.28 cm^2**
R24	**5**	**8.8 cm**	**19.1 cm**	**7**	**17.25 cm^2**
R35	**6**	**17 cm**	**19.2 cm**	**7**	**18.52 cm^2**
R40	**7**	**15 cm**	**26.5 cm**	**6**	**18.15 cm^2**
R50	**8**	**12.5 cm**	**34 cm**	**8**	**20.07 cm^2**
R53	**9**	**8 cm**	**38.6 cm**	**7**	**13 cm^2**
Control	**10**	**9.5 cm**	**21 cm**	**6**	**19.68 cm^2**

CONCLUSION

On the basis of the results of this preliminary study it may be said that these rhizobacterial isolates should be subjected to field tests for evaluation of their performance to improve plant growth in *Spinacia oleracea* to eventually develop them into soil inoculants.

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