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Full Length Research Paper

Effect of plant growth promoting rhizobacteria on root morphology of Safflower (*Carthamus tinctorius* L.)

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Rooting characteristics significantly affect the water-use patterns and acquirement of nutrient for any plant species. Plant growth promoting rhizobacteria improve the plant growth by a variety of ways like the production of phytohormones, nitrogen fixation, phosphate solubilization and improvement in root morphology etc, and are also useful in cutting down the cost of chemical fertilizers. The present investigation was carried out to determine the comparative effect of plant growth promoting rhizobacteria (PGPR), *Azospirillum brasilense*, *Azotobacter vinelandii* and *Pseudomonas stutzeri,* either alone or in combination with different doses of chemical fertilizers [full dose (Urea at 60 kg ha^{-1} and DAP at 30 kg ha^{-1}), half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}) and quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1})] on root morphology and root distribution pattern of safflower (*Carthamus tinctorius* L.) viz. cvv. Thori and Saif-32 in the soil. The PGPR were applied as seed inoculation at 10^{6} cells/ml prior to sowing. *P. stutzeri* either alone or in combination with full dose of chemical fertilizers, was highly effective in increasing the root area in cv. Saif-32, whereas, the percent increase due to *A. brasilense* was comparable to that of treatment with full dose of chemical fertilizers. *P. stutzeri* inoculation resulted in significantly higher root length in both the cultivars. Significantly, higher root width (54%) of cv. Thori was observed in treatment receiving inoculation with *A. vinelandii* and supplemented with half dose of chemical fertilizers, whereas maximum root width of cv. Saif-32 was recorded in treatment supplemented with half dose of chemical fertilizers. It is inferred that PGPR inoculation especially those of *A. brasilense* and *P. stutzeri* either alone and more so in combination with half dose of chemical fertilizers, are highly effective in improving root morphology and growth in safflower.

Key words: Root area, safflower, plant growth promoting rhizobacteria (PGPR), root growth, chemical fertilizers.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are free-living soil-borne bacteria that colonize the rhizosphere and when applied to seed or crops, enhance the growth of plants (Kloepper et al., 1980). They have been reported to increase the percentage seed germination, emergence, shoot growth, root growth, total biomass of the plants, induce early flowering and increase the grain yield (Van-Loon et al., 1998; Ramamoorthy, 2001). These improvements in growth attributes of plants caused by PGPR are brought about due to their potential of nitrogen fixation and production of phytohormones like auxin, gibberellins, cytokinin, and phosphate solubilization, resulting in the availability of nutrients to plants and increase in roots permeability (Enebak and Carey, 2000).

As a primary target, root is the organ that shows the first stimulating bacterial effects. This was particularly remarkable in plants inoculated with ***Azospirillum*** spp. (Okon, 1985). Plant growth promoting rhizobacteria have been reported for altering the root architecture of plants (Mantelin et al., 2006). Auxin, a phytohormone, is considered to positively affect the growth of roots. However, the auxin mutants were found to retain the capacity to elongate their root hairs when inoculated by PGPR (Desbrosses et al., 2009).

Previous experiments showed that inoculation with ***Azospirillum*** markedly improved yields, which were accompanied by better water and mineral uptake and remarkable positive alterations in the growth and

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morphology of root (Creus et al., 2004; Dobbelaere et al., 2001). The mechanisms involved in root distribution can be measured by quantifying root length, diameter and surface area (Gamalero et al., 2002). Therefore, an increase in the degree of branching of roots associated with improved root morphology would contribute to a better plant growth and ultimately greater yields.

Safflower has been grown from a long of time for its colorful petals, which was used in food coloring and flavoring agent, as a source of vegetable oils and also for preparing textile dye in the Far East, Central and Northern Asia and European Caucasian (Esendal, 2001). Regarding the human health and nutritional physiology, vegetable oil is one of the fundamental components in foods that have important functions. Consumers have demanded healthier oils, naturally low in saturated fats. From this perspective, safflower has received a lot of importance as a source of vegetable oil. The seeds of safflower contain 35 to 50% oil, 15 to 20% protein and 35 to 45% hull fraction (Rahamatalla et al., 2001). This plant is considered as a drought tolerant crop, which is capable of obtaining moisture from levels not available to the majority of crops (Weiss, 2000). Safflower can also be grown successfully on soil with poor fertility and in areas with relatively low temperatures (Koutroubas and Papakosta, 2005). Safflower is also being used as a source of alternative fuel (biodiesel) these days.

The current investigation was therefore aimed to compare the effect of PGPR, either alone or in combination with different doses of chemical fertilizers, on root growth and morphology of safflower.

MATERIALS AND METHODS

The experiment was carried out in complete randomized design (CRD) at the Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Certified seeds of Safflower cv. Thori and Saif 32 were obtained from National Agriculture Research Centre (NARC), Islamabad. The seeds were sown in plastic pots (11 × 8 cm^2) filled with autoclaved (temperature 121°C and pressure 15 Pascal) loamy soil and sand in 1:1 ratio under controlled sterilized conditions in a growth chamber (16 h light period at 24°C, 8 h dark period at 18°C and 60% relative humidity) and watered with autoclaved sterilized water. Seedlings were harvested after one month of sowing.

Method of seed inoculation

The seeds of safflower were surface sterilized with 95% ethanol followed by soaking in 10% clorox with intermittent stirring for 5 min and subsequently washed three times with sterilized distilled water. The ***Azospirillum brasilense*** (isolated from rhizosphere of wheat), ***Azotobacter vinelandii*** Khsr1 (isolated from roots of ***Chrysopogon aucheri***) and ***Pseudomonas stutzeri*** Khsr3 (isolated from the roots of ***Solanum surattense***) was applied as seed inoculation at10^6 cells/ml and the number of bacterial colonies/seed were measured 4×10^5.

For inoculum preparation, 24 h old fresh cultures were inoculated in 100 ml broth of Luria-Bertani media (LB), kept on shaker (Excell E24, New Brunswick Scientific Incubator shaker Series, New Gersey, USA) for 72 h at 120 rpm and centrifuged for 10 min at 10,000 rpm. Supernatant was discarded and pellet was diluted with distilled water up to 100 ml and then optical density was measured at 600 nm wavelength. Sterilized seeds were soaked in culture for 4 h and then sown.

Chemical fertilizers were applied in the form of urea (source of nitrogen) and diammonium phosphate (DAP) (source of phosphorus) at 60 kg ha^{-1} and 30 kg ha^{-1}, respectively. The fertilizers were applied at the time of sowing in the form of aqueous solution. The mode of application / treatments is shown in Table 1.

Parameters studied

The plants were harvested after one month of sowing and root morphology was determined using 'Root Law' (Washington State University) software. The phytohormone production (IAA and GA etc.) and the capabilities of the respective PGPR viz. ***A. brasilense, A. vinelandii*** and ***P. stutzeri*** were demonstrated by Ilyas and Bano (2010), Naz et al. (2009) and Naz and Bano (2010), respectively.

Statistical analysis

The data were analyzed statistically by Statistix version 8.1 technique and comparison among mean values of treatments was made by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

A dynamic root system is important for regulating the availability of water to the plant (Toorchi et al., 2002). This spatial allocation of roots and their biomass in the soil are the greater determinants of the ability of crops to gain the nutrients and water essential for growth (Li et al., 2006). During the current investigation, it was observed that in cv. Thori, all the treatments significantly increased the root area; however, maximum increase (90, 91 and 90%) was recorded in ***P. stutzeri*** alone when supplemented with half and quarter doses of chemical fertilizers, respectively (Figure 1). Nevertheless, quarter dose of chemical fertilizers and inoculation with ***A. brasilense*** showed similar results (88 and 87%) as compared to untreated control. These results indicate the positive role of PGPR in enhancing root growth, which may counteract the fertilizer effect. However, the inoculation of ***A. brasilense*** along with application of half and quarter doses of chemical fertilizers markedly improved (79 and 61%) the root area than un-inoculated control. The impact of ***A. vinelandii*** and ***P. stutzeri*** co-inoculation was more pronounced (86%) than that of ***A. brasilense*** and ***A. vinelandii*** co-inoculation, which was 51% greater with both treatments, compared with untreated control, respectively. In case of cv. Saif-32, significant increase in root area was observed in almost all the treatments except ***A. brasilense*** + quarter dose of chemical fertilizers. Whereas, inoculation with ***P. stutzeri*** along with full dose of chemical fertilizers exhibited maximum (47%) increase in root area. Furthermore, ***A. brasilense*** and ***A. vinelandii*** significantly increased the root area by 33 and 39% when inoculated with half dose of chemical

Table 1. Treatment of seeds of safflower.

S/N	Treatment	Abbreviation
1	Control (Without inoculation and without chemical fertilizers)	C
2	Chemical fertilizers full dose (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1})	CFF
3	Chemical fertilizers half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1})	CFH
4	Chemical fertilizers quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1})	CFQ
5	***Azospirillum brasilense***	SP
6	***A. brasilense*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1})	SPF
7	***A. brasilense*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1})	SPH
8	***A. brasilense*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1})	SPQ
9	***Azotobacter vinelandii***	BT
10	***A. vinelandii*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1})	BTF
11	***A. vinelandii*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1})	BTH
12	***A. vinelandii*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1})	BTQ
13	***A. brasilense*** + ***A. vinelandii***	SPBT
14	***Pseudomonas stutzeri***	P
15	***P. stutzeri*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1})	PF
16	***P. stutzeri*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1})	PH
17	***P. stutzeri*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1})	PQ
18	***P. stutzeri*** + ***A. vinelandii***	P BT

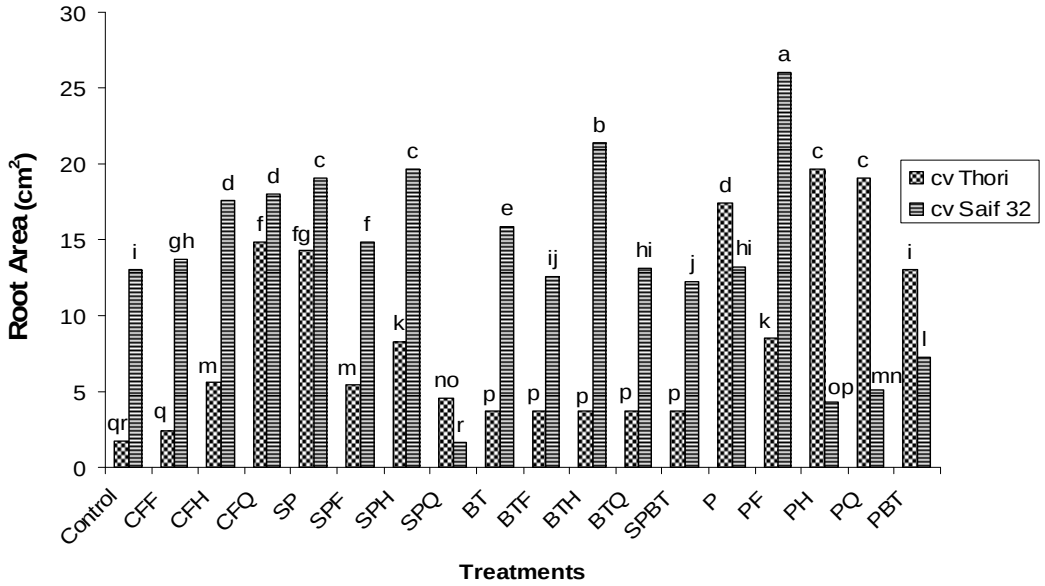

Figure 1. Effect of ***A. brasilense***, ***A. vinelandii***, ***P. stutzeri*** and chemical fertilizers on root area (cm^3) of safflower viz. cvv. Thori and Saif-32. The experiment was carried out in pots with three replicates. All such means which share a common English letter are similar; otherwise differ significantly at P<0.05. C, Control; CFF, chemical fertilizers full dose (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); CFH, chemical fertilizers half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); CFQ, Chemical fertilizers quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SP: ***A. brasilense***, SPF, ***A. brasilense*** +full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); SPH, ***A. brasilense*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); SPQ, ***A. brasilense*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); BT, ***A. vinelandii***; BTF, ***A. vinelandii*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); BTH, ***A. vinelandii*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); BTQ, ***A. vinelandii*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SPBT, ***A. brasilense*** + ***A. vinelandii***; P, ***P. stutzeri***; PF, ***P. stutzeri*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{1}); PH, ***P. stutzeri*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); PQ, ***P. stutzeri*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); PBT, ***P. stutzeri*** + ***A. vinelandii***

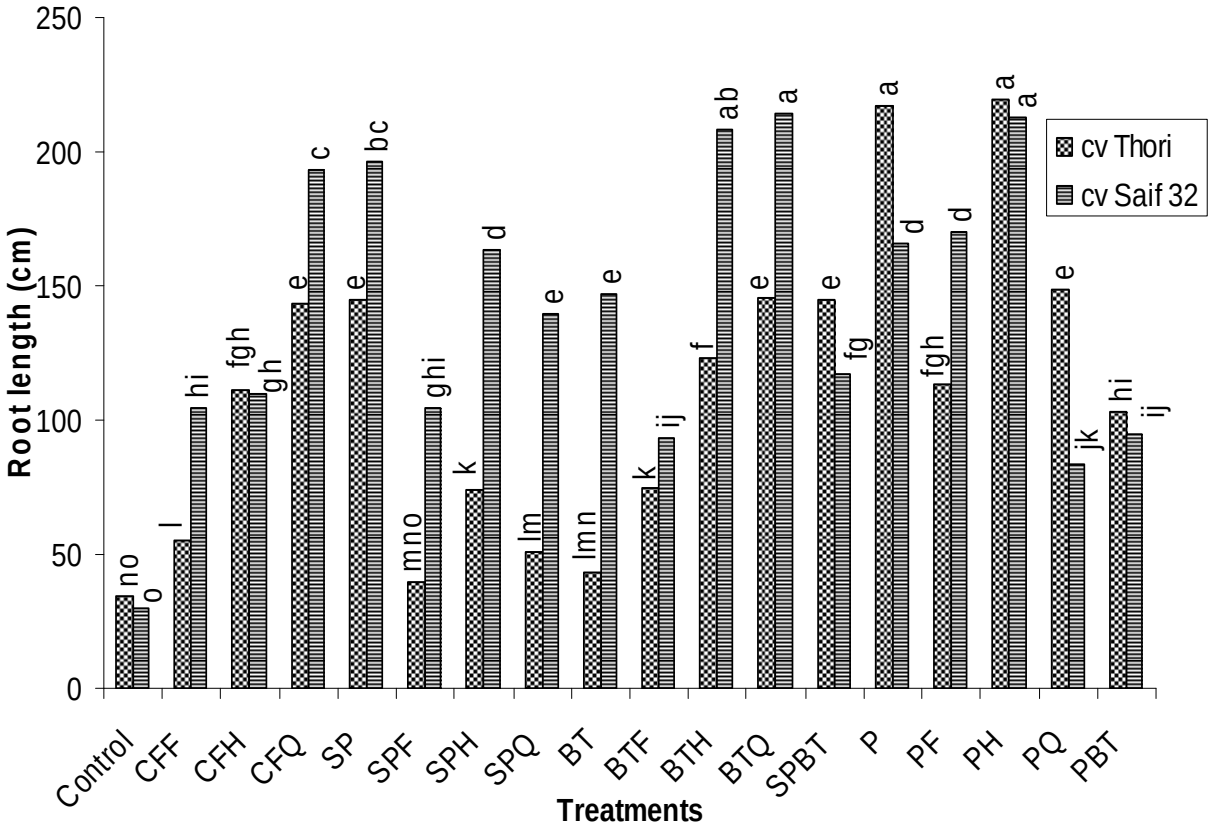

Figure 2. Effect of ***A. brasilense***, ***A. vinelandii, P. stutzeri*** and chemical fertilizers on root length (cm) of safflower viz. cvv. Thori and Saif-32. The experiment was carried out in pots in three replicates. All such means which share a common English letter are similar; otherwise differ significantly at P<0.05. C, Control; CFF, chemical fertilizers full dose (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); CFH, chemical fertilizers half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); CFQ, chemical fertilizers quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SP: ***A. brasilense***, SPF, ***A. brasilense*** +full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); SPH, ***A. brasilense*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); SPQ, ***A. brasilense*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); BT, ***A. vinelandii***; BTF, ***A. vinelandii*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); BTH, ***A. vinelandii*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); BTQ, ***A. vinelandii*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SPBT, ***A. brasilense*** + ***A. vinelandii***; P, ***P. stutzeri***; PF, ***P. stutzeri*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{1}); PH, ***P. stutzeri*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); PQ, ***P. stutzeri*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); PBT, ***P. stutzeri*** + ***A. vinelandii***.

fertilizers. ***A. brasilense*** treatment improved 30 and 17% root area as compared to ***P. stutzeri*** and ***A. vinelandii***, respectively.

Results presented in Figure 2 showed that root length was higher in cv. Saif-32 as compared to cv. Thori. It was found that root length was significantly enhanced by all treatments in both the varieties. Among chemical fertilizer treatments (Figure 4), maximum root length was recorded in treatment supplemented with quarter dose of chemical fertilizers in both the cultivars. It was also observed that root length of safflower was gradually increased by decreasing the dose of chemical fertilizers. ***A. brasilense*** improved the root length of both cultivars; maximum response being shown by cv. Saif-32 (Figure 9). In cv. Thori, the inoculation effects of ***A. brasilense*** (Figure 5) were more pronounced when applied alone rather than its application along with different doses of chemical fertilizers. In the same cultivar, inoculation effects of ***A. brasilense*** on root length were more pronounced (70%) than ***A. vinelandii*** (Figure 6). The co-inoculation of ***A. brasilense*** and ***A. vinelandii*** caused 76% improvement in root length than un-inoculated control, whereas inoculation with ***P. stutzeri*** exhibited 84% increase in root length. However, the application of chemical fertilizers along with ***P. stutzeri*** (Figure 7) did not improve further the root length. The treatment having co-inoculation of ***A. brasilense*** and ***A. vinelandii*** showed 29% higher root length than co-inoculation of ***P. stutzeri*** and ***A. vinelandii***. In cv. Saif-32, the impact of ***A. brasilense*** on root length was greater than cv. Thori, having 84% higher root length than untreated control. The inoculation with ***A. brasilense*** along with quarter dose of chemical fertilizers showed

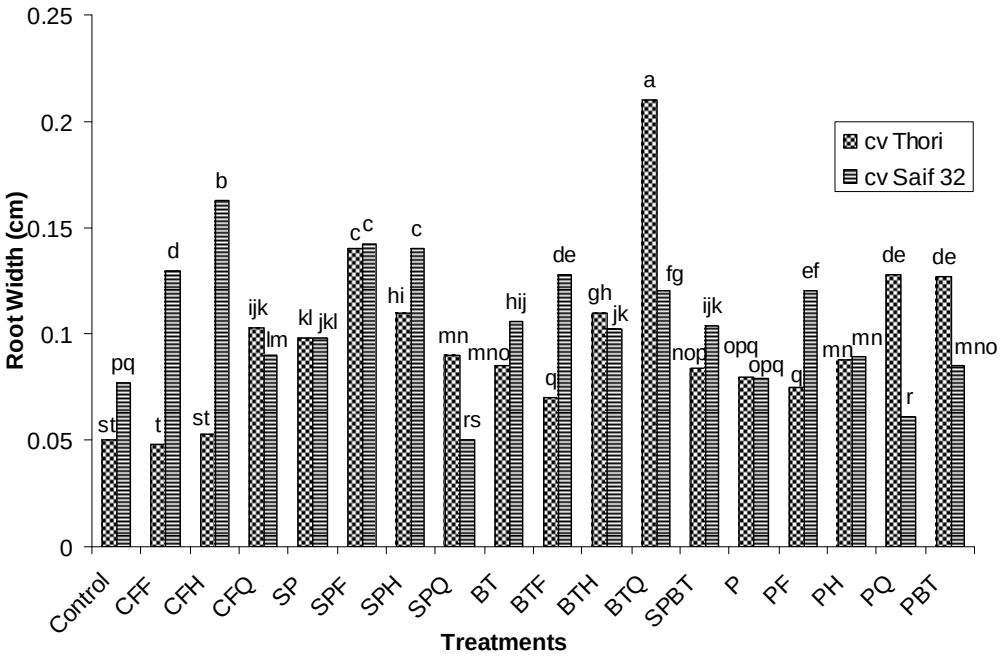

Figure 3. Effect of ***A. brasilense***, ***A. vinelandii***, ***P. stutzeri*** and chemical fertilizers on root width (cm) of safflower viz. cvv. Thori and Saif-32. The experiment was carried out in pots with three replicates. All such means which share a common English letter are similar; otherwise differ significantly at $P<0.05$. C, Control; CFF, chemical fertilizers full dose (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); CFH, chemical fertilizers half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); CFQ, chemical fertilizers quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SP: ***A. brasilense***, SPF, ***A. brasilense*** +full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); SPH, ***A. brasilense*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); SPQ, ***A. brasilense*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); BT, ***A. vinelandii***; BTF, ***A. vinelandii*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); BTH, ***A. vinelandii*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); BTQ, ***A. vinelandii*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SPBT, ***A. brasilense*** + ***A. vinelandii***; P, ***P. stutzeri***; PF, ***P. stutzeri*** + full dose of chemical fertilizers (Urea 60 kg ha^{-1} and DAP 30 kg ha^{1}); PH, ***P. stutzeri*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); PQ, ***P. stutzeri*** + quarter dose of chemical fertilizers (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); PBT, ***P. stutzeri*** + ***A. vinelandii***.

significantly greater (78%) root length than un-inoculated control (Figure 9). Perhaps full dose of chemical fertilizer + PGPR inhibits root length**.** The inoculation with ***A. vinelandii*** (Figure 10) caused 80% increase in root length as compared to untreated control; which was 25% lower than impact of ***A. brasilense***. It was observed that root length of cv.Saif-32 was further improved by application of quarter dose of chemical fertilizers (Figure 8). It was also observed that effect of ***P. stutzeri*** on root length (Figure 11) was more pronounced (11%) than inoculation with ***A. vinelandii***. The results show that impact of co-inoculation of ***A. brasilense*** and ***A. vinelandii*** on root length was greater (19%) than co-inoculation of ***P. stutzeri*** and ***A. vinelandii***.

Roots with larger diameters (root width) result in greater biomass of root (Eissenstat and Yanai, 2002), whereas roots with smaller diameters result in root system with greater surface area (Waisel and Eshel, 2002). Current results in Figure 3 showed that all the treatments positively affected the root width. Maximum (76%) root width was recorded in treatment having inoculation with ***A. vinelandii*** and supplemented with quarter dose of chemical fertilizers in cv. Thori. ***A. brasilense*** supplemented with full and half dose of chemical fertilizers significantly increased (64 and 54%) root width as compared to control. The magnitude of increase in root width by ***A. vinelandii*** supplemented with half dose of chemical fertilizers and ***P. stutzeri*** supplemented with quarter dose of chemical fertilizers was 54 and 61% as compared to the control. ***A. vinelandii*** and ***P. stutzeri*** co-inoculation improved (34%) root width as compared to ***A. brasilense*** and ***A. vinelandii*** co-inoculation (Figure 3). In the case of cv. Saif-32, maximum increase (52%) in root width was recorded in half dose of chemical fertilizers followed by full dose of chemical fertilizers (40%). However, 45% increase was recorded in ***A. brasilense*** supplemented with both full and half dose of chemical fertilizers. Similarly, ***A. vinelandii***

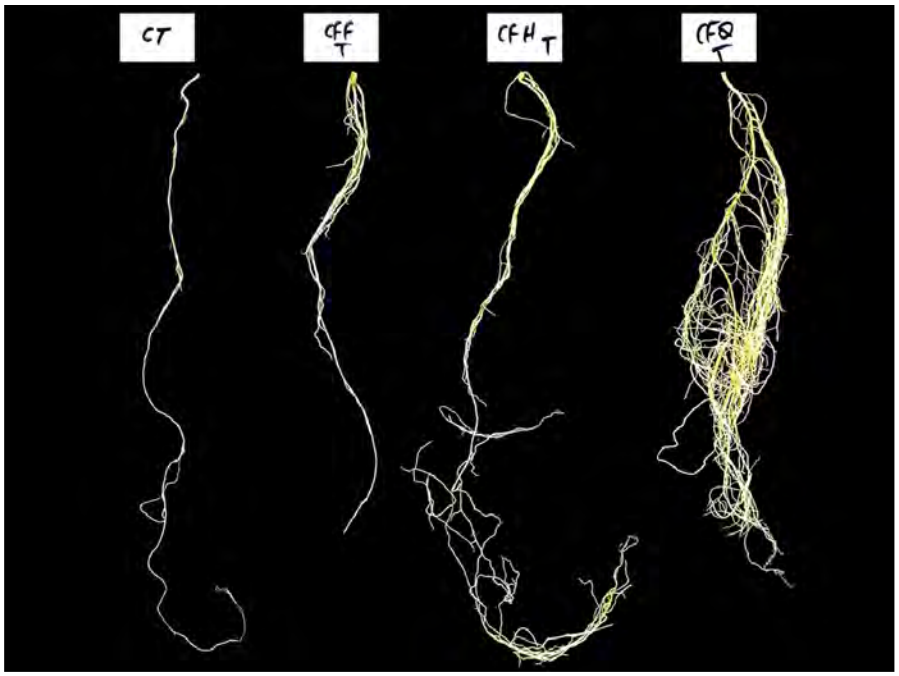

Figure 4. Morphological variations shown in root architecture of safflower cv. Thori under various treatments of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. C, Control ((without inoculation and chemical fertilizers); CFF, chemical fertilizers full dose (Urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); CFH, chemical fertilizers half dose (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); CFQ, chemical fertilizers quarter dose (Urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}).

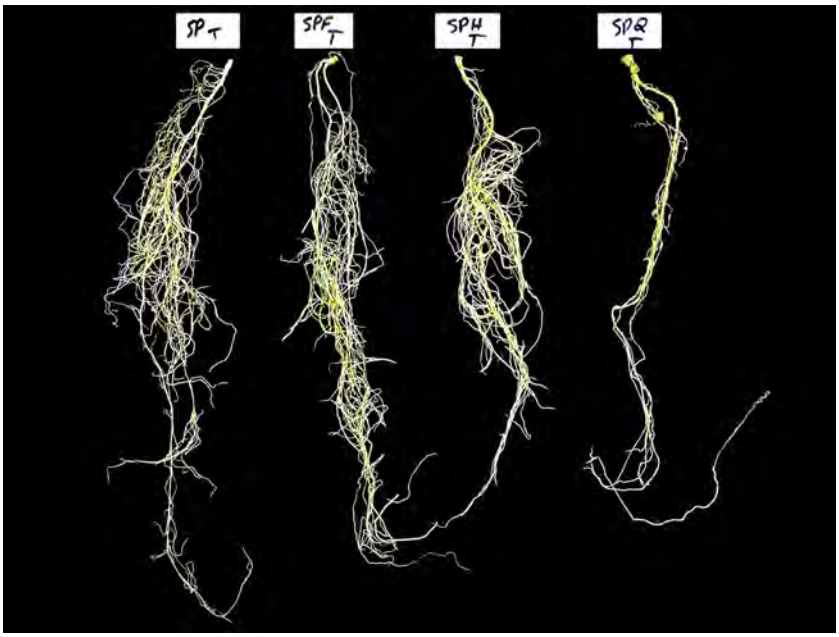

Figure 5. Morphological variations in root of safflower cv. Thori under various treatments of ***A. brasilense*** alone and in combination with different doses of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. SP, ***Azospirillum brasilense***; SPF, ***A. brasilense*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); SPH, ***A. brasilense*** + half dose of chemical fertilizers (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); SPQ,***A. brasilense*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}).

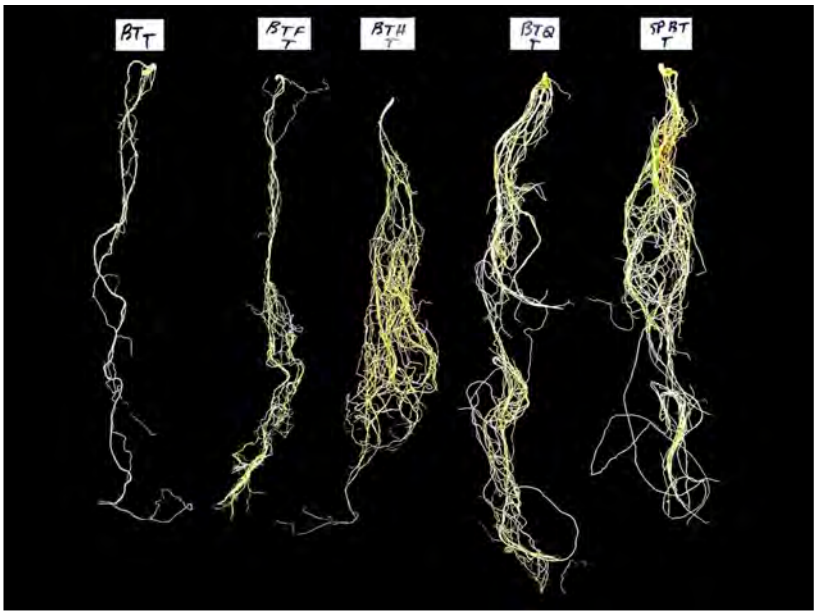

Figure 6. Morphological variations in root of safflower cv. Thori under various treatments of ***A. vinelandii*** alone and in combination with different doses of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. BT, ***Azotobacter vinelandii***; BTF, ***A. vinelandii*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); BTH, ***A. vinelandii*** + half dose of chemical fertilizers (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); BTQ, ***A. vinelandii*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SPBT, ***A. brasilense***+***A. vinelandii.***

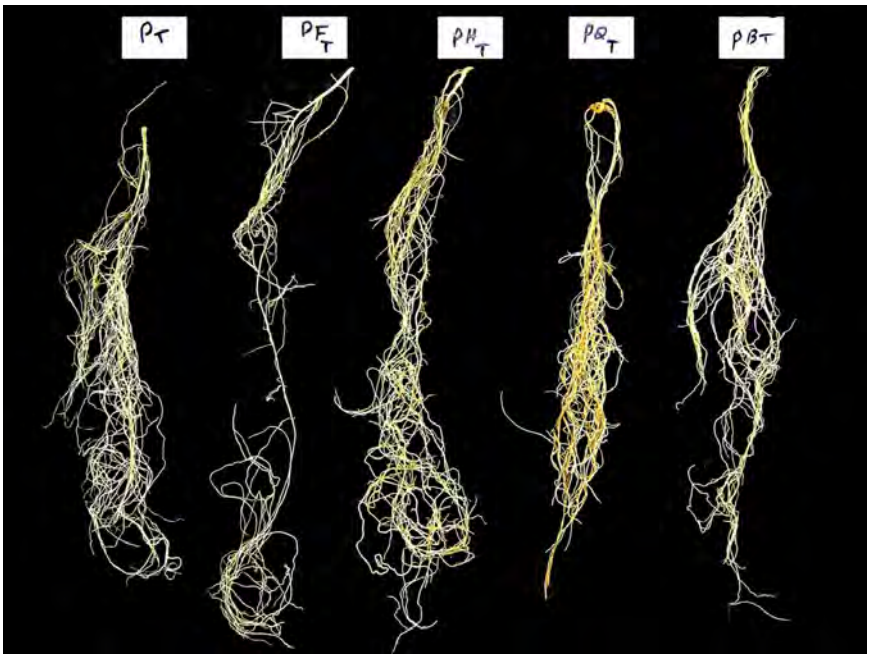

Figure 7. Morphological variations in root of safflower cv. Thori under various treatments of ***P. stutzeri*** alone and in combination with different doses of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. P, ***Pseudomonas stutzeri***; PF, ***P. stutzeri*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{1}); PH, ***P. stutzeri*** + half dose of chemical fertilizers (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); PQ, ***P. stutzeri*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); PBT, ***P. stutzeri*** + ***A. vinelandii.***

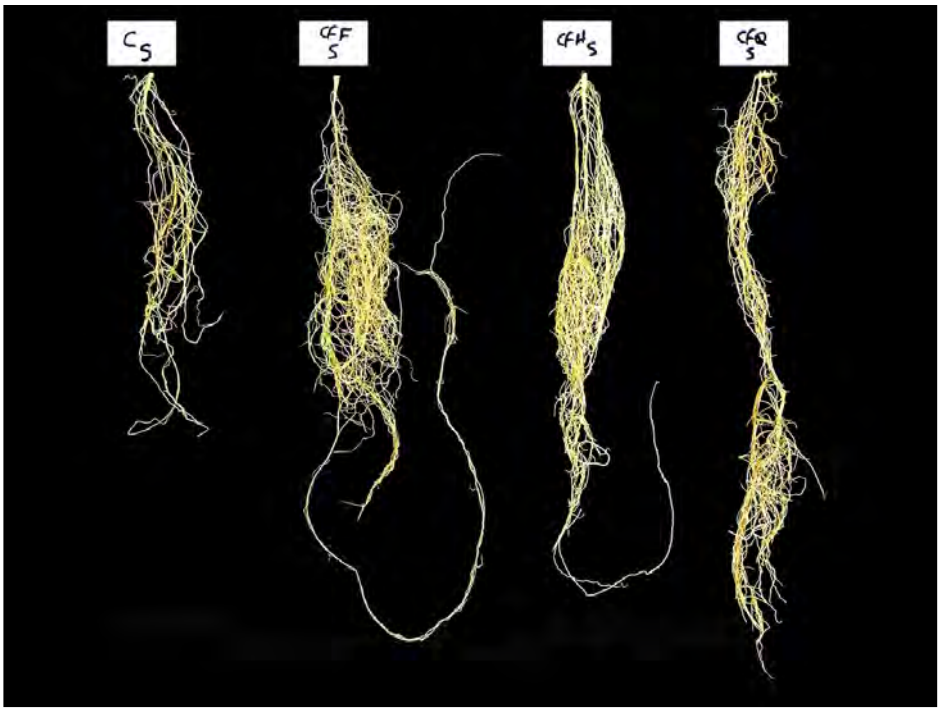

Figure 8. Morphological variations shown in root architecture of safflower cv. Saif-32 under various treatments of chemical fertilizers (Urea and DAP). The plants were harvested after one month of sowing. C, Control (without inoculation and chemical fertilizers); CFF, chemical fertilizers full dose (urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); CFH, chemical fertilizers half dose (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); CFQ, chemical fertilizers quarter dose (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}).

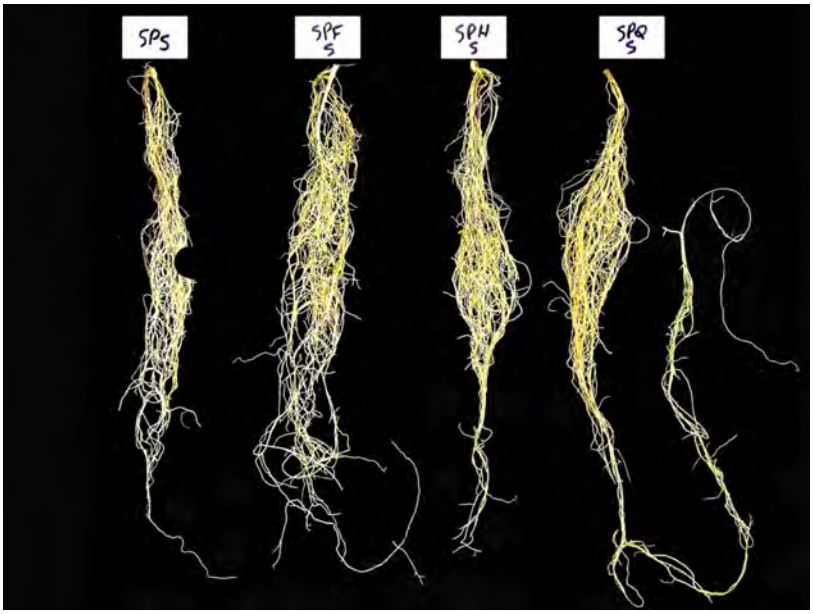

Figure 9. Morphological variations in root of safflower cv. Saif-32 under various treatments of ***A. brasilense*** alone and in combination with different doses of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. SP, ***Azospirillum brasilense***; SPF, ***A. brasilense*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); SPH, ***A. brasilense*** + half dose of chemical fertilizers (Urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); SPQ, ***A. brasilense*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{1}).

Figure 10. Morphological variations in root of safflower cv. Saif-32 under various treatments of ***A. vinelandii*** alone and in combination with different doses of chemical fertilizers (Urea and DAP). The plants were harvested after one month of sowing. BT, ***Azotobacter vinelandii***; BTF, ***A. vinelandii*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{-1}); BTH, ***A. vinelandii*** + half dose of chemical fertilizers (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); BTQ, ***A. vinelandii*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); SPBT, ***A. brasilense*** +***A. vinelandii.***

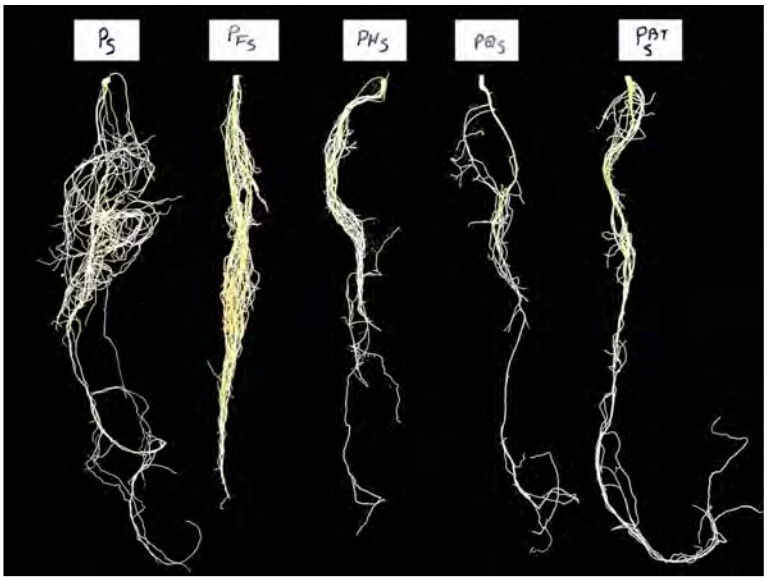

Figure 11. Morphological variations in root of safflower cv. Saif-32 under various treatments of ***P. stutzeri*** alone and in combination with different doses of chemical fertilizers (urea and DAP). The plants were harvested after one month of sowing. P, ***Pseudomonas stutzeri***; PF, ***P. stutzeri*** + full dose of chemical fertilizers (urea 60 kg ha^{-1} and DAP 30 kg ha^{1}); PH, ***P. stutzeri*** + half dose of chemical fertilizers (urea 30 kg ha^{-1} and DAP 15 kg ha^{-1}); PQ, ***P. stutzeri*** + quarter dose of chemical fertilizers (urea 15 kg ha^{-1} and DAP 7.5 kg ha^{-1}); P BT: ***P. stutzeri*** + ***A.***

alone and in combination with full, half and quarter dose of chemical fertilizers caused 27, 39, 24 and 35% increase as compared to un-inoculated control. ***P. stutzeri*** supplemented with full dose of chemical fertilizers exhibited 35% increase in root width as compared to the control. Moreover ***A. brasilense*** and ***A. vinelandii*** co-inoculation resulted in 52% increase in root width as compared to ***A. vinelandii*** and ***P. stutzeri*** co-inoculation.

The beneficial effects of PGPR on root growth have been reported in wheat (Levanony and Bashan, 1989). Previous studies showed that plant growth promotion activity of ***Azospirillum*** was primarily related to its impact on root growth and morphology (Okon, 1985). Similarly, PGPR inoculation caused the production of lengthy root hairs, stimulated the production of lateral roots, and improved the root diameter and area respectively (Creus et al., 2004; Dobbelaere et al., 1999). Maximum root diameter was recorded in treatment having being inoculated with ***A. vinelandii***, establishing the production of root system with greater biomass in cv. Thori, whereas in the same variety, ***A. brasilense*** produced roots with small width, indicating its potential role in improving the root surface area. ***P. stutzeri*** was highly effective in improving the root area and length in safflower. These results are in agreement with previous findings of Egamberdieva and Hoflich (2003) whose report showed

that inoculation of wheat with ***Pseudomonas*** caused significant increase in root length and growth.

The production of phytohormones namely auxins, cytokinins, and gibberellins, is the most commonly invoked mechanism of plant growth promotion exerted by PGPR (Garcı´a de Salamone et al., 2001). Among them, auxins are thought to play the major role in the development of root system. The PGPR investigated during current investigation have been reported for their production of phytohormones in the culture medium (Ilyas and Bano, 2010**;** Naz et al., 2009; Naz and Bano, 2010), which might have contributed to the improvement of the rooting system of safflower. ***Pseudomonas*** and ***Azospirillum*** has the potential to synthesize plant hormones that can replace indole acetic acid (IAA) to stimulate root growth in wheat and vegetable soybean, respectively (Egamberdieva, 2010; Molla et al., 2001). Dobbelarere et al. (1999) suggested that secretions of plant growth promoting substances such as auxins, gibberellins and cytokinins by the bacteria seem to be responsible for these effects. Desbrosses et al. (2009) also reported that auxin mutants were found to retain the capacity to elongate their root hairs when inoculated by PGPR. The inoculation effects of ***A. brasilense*** along with half dose of chemical fertilizers were greater on root area than the application of full dose of chemical fertilizers and without inoculation of this PGPR strain. These results are in agreement with previous findings of Okon and Kapulnik (1986) that root surface area and length were increased due to ***Azospirillum*** inoculation. This stimulatory effect of PGPR inoculation might be due to increased rate of cell division as reported in wheat's root (Levanony and Bashan, 1989).***A. vinelandii*** markedly increased the root diameter in safflower. This microbe has been reported for the production of auxin and cytokinin in the culture medium (Naz et al., 2009), which might have contributed to increase in the root diameter in safflower because the beneficial effects of auxin on root diameter have been reported earlier (Christopher et al., 2004). It was observed that cv. Saif-32 was more responsive to ***Azospirillum*** inoculation than cv.Thori. These results are also in agreement with previous findings that those effects of ***Azospirillum*** on root growth are dependant on the type of cultivar inoculated (Vande-Broek et al., 2000). Similarly, Chanway et al. (1988) observed that the extent of positive effects of the bacteria on plant growth varied with the species or variety of the host plant.

Conclusion

It is inferred that ***A. brasilense*** and ***P. stutzeri*** are effective PGPR strains that improved the root morphology of safflower as evidenced by their impact on root area, length and diameter, respectively. It is therefore recommended that inoculation with these PGPR, either alone or more so in combination with half and quarter doses of chemical fertilizers, could be highly beneficial in improving the water and nutrient availability to safflower plants. Moreover, the impact of selected PGPR strains was different on two safflower cultivars. Therefore, before the selection of PGPR strains for safflower there should be screening of cultivars that benefit from association with these beneficial microbes.

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