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# Influence of zoysiagrass rhizosphere fungal isolates on growth and yield of soybean plants

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**Among 21 rhizosphere fungi tested, eight sterile fungi and one *Trichoderma* isolate (GT2-1) from zoysiagrass rhizosphere promoted the overall growth of soybean varieties when grown in the greenhouse. Out of nine effective isolates, GS7-4, GS8-2, GS8-3, GU23-3 (all sterile fungi) and GT2-1 (*Trichoderma* sp.) promoted plant growth and increased yield of Toyosuzu (variety 1) significantly, while GS8-3, GS10-1, GS10-2 (sterile fungi), and GT2-1 significantly caused plant growth promotion and yield increase of Kitamusume (variety 2). Among these efficient isolates, GS8-3 and GS10-2 induced considerable and consistent increases in length, biomass and yield of plants of varieties 1 and 2, respectively. In the field, however, only GS8-3 and GU23-3 among seven selected isolates, induced consistent and significant increases in plant growth and yield of varieties 1 and 2, while the ability of other isolates decreased. The plant growth promotion by these isolates in the field followed a similar trend to that in the greenhouse, but the effect was less marked. Some isolates which were effective in the greenhouse were less effective in the field. The degree of growth promotion by different isolates depended on the variety of soybean. The nutrient condition of soils used in experiments also seemed to play a vital role, since notable growth promotion by these isolates was observed in nutrient-depleted soil.**

Key Words——barley grain inoculum; growth promotion; soybean; yield components; zoysiagrass rhizosphere.

## Introduction

The beneficial effect of bacteria and fungi in the rhizosphere in terms of plant growth promotion and biological control has attracted renewed interest among researchers. Much of the literature relate to plant growth promoting rhizobacteria whose effects are also exploited to control diseases and induce resistance in plants (Liftshitz et al., 1987; Chanway and Nelson, 1991; Wei et al., 1991; van Peer and Schippers, 1992). Among fungi, species of *Trichoderma* and *Rhizoctonia* have been found to promote plant growth as well as to effect biocontrol (Sneh et al., 1986; Windham et al., 1986; Baker, 1991; Kleifeld and Chet, 1992). Sterile fungi isolated from rhizosphere of wheat (*Triticum aestivum* L.), have also proved effective both as plant growth promoters and biocontrol agents (Dewan and Sivasithamparam, 1989a; Narita and Suzui, 1991).

Previous research in this laboratory revealed that among several fungal isolates from rhizospheres of cultivated and non-cultivated crops, those isolated from zoysiagrass (*Zoysia tenuifolia* Willd. ex Trin.) rhizosphere promoted growth of cucumber (*Cucumis sativus* L.), radish (*Raphanus sativus* L.), tomato (*Lycopersicon esculentum* Mill.) and wheat plants and also bentgrass (*Agrostis palustris* Huds.) (Hyakumachi et al., 1992, 1993). Studies of plant growth promotion by rhizosphere fungi were restricted to the four-week stage (Dewan and Sivasithamparam, 1989a; Hyakumachi et al., 1993; Shankar et al., 1993). Preliminary studies on growth promotion in wheat and soybean (*Glycine max* (L.) Merr.) also revealed that certain fungal isolates from turfgrass rhizosphere, particularly from zoysiagrass rhizosphere (ZR), enhanced growth through the seed harvest stage in the greenhouse (Shivanna et al., 1993). Later, ZR isolates were also shown to promote growth and increase yield of wheat plants in the field (Shivanna et al., 1994). We were interested to study whether such growth promotion by ZR isolates would also occur in a dicotyledonous crop like soybean beyond the four-week stage, until the seed harvest stage in the field. Hence, the present study was taken up.

## Materials and Methods

**Description of test isolates and their preparation** Twenty-one fungal isolates were collected from rhizospheres of zoysiagrass and classified into three groups: 'Sporulating' (species of *Penicillium* (isolates E-4 and F-3) and *Trichoderma* (isolate GT2-1), 'Sterile I' (GS series, 13 isolates) and 'Sterile II' (GU series, 5 isolates). Sterile I and II isolates had septa and failed to produce asexual/sexual spores in any of the semi-synthetic and synthetic media

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tested. However, most of them produced chlamydospore-like structures. Sterile I isolates produced aerial or settling, cottony-white to pinkish mycelia and no pigments or pale pink to pinkish pigments in the medium. Certain isolates produced a dirty-white, thick mycelial mat on the surface of the medium, often with mycelial clumps. Sterile II isolates produced aerial or matty, light-black to blackish mycelia on the media and did not produce any pigment. Their growth rates on media differed with isolates. Sterile I and II isolates did not produce any clamp connections and did not show any characteristics of mycorrhizae. Isolates of GS and GU series will be referred to as 'sterile', unless specifically mentioned.

All fungal isolates were mass cultured on autoclaved barley grains (Shivanna et al., 1994). The barley grain inocula of ZR isolates were tested for their viability and growth of fungal isolates on PDA before addition to the soil.

**Greenhouse assay** Barley grain inocula (1%, w/w) of the fungal isolates were used to amend separately a 1 : 1 mixture of air-dried soil (unsterilized Gifu Univ. Campus field soil, brown loam, pH 6.5–7, carbon 0.27%, total nitrogen 0.048%, soluble nitrogen 1.6 mg/kg, total phosphorus 0.11% and soluble phosphorus 0.022 mg/kg) and river sand. Pots (19 cm diam × 19 cm depth) containing 3000 g of soil-sand-inoculum mixture were sown with five seeds of each of two varieties of soybean, Toyosuzu (var. 1) and Kitamusume (var. 2). In each treatment, there were at least three replicates with five plants in each replicate. The soil-sand mixture unamended and amended with autoclaved, uninfested barley grain were considered as untreated and treated controls, respectively. The pots were arranged in a split-plot design with 21 treatments as main plot factors and two varieties as sub-plot factors. The experiment was conducted during the period May-September in 1992 and 1993. All treatments received natural day light intensity of spring/summer and frequent watering. An average temperature of 28°C and RH of 80% prevailed during the period.

**Field trial** The field soil (Gifu Univ. experimental plot, Kakamigahara, brown loam, pH 6–7.5, carbon 6.7%, total nitrogen 0.5%, soluble nitrogen 2.2 mg/kg, total phosphorus 0.96% and soluble phosphorus 0.05 mg/kg) was prepared and left uncultivated for three months. The experiment was laid out in a split-plot design and conducted during May-September, 1992–93, consisting of nine treatment subplots (10 m long × 5 m wide), each containing four rows (each row 10 m long; 100 cm between rows). Seven selected isolates, GS6-1, GS6-2, GS8-2, GS8-3, GS10-1, GS10-2, and GU23-3, were tested in comparison with the treated and untreated controls. The barley grain inoculum of each fungus or autoclaved, unifested barley grains were placed inside the furrow at intervals of 1 cm (1000 colonized barley grains or ca. 20 g per row); the same furrows were also sown with 50 seeds (20 cm between seeds) of each of the two varieties used in the greenhouse assay and covered with 1 cm of soil. Fifteen plants selected randomly formed a replicate and three such replicates were maintained for each treatment. The plants received a total rainfall of 22 cm, and an average temperature of 25°C and 75% RH. The soil was not supplemented with fertilizers; herbicides were not applied. Hand weeding was done fortnightly.

**Analysis of growth and yield components** Plants were tested for the enhancement of length and dry biomass at the ninth week (pod maturation) stage. Such yield components as the number of pods, pod (intact pods with seeds) dry biomass, number of seeds and seed (only seeds) dry biomass were also determined at the tenth week (seed harvest) stage. The shoot portion was cut and height was measured. The dry biomass of plants, pods and seeds were determined after drying them at 100°C for 24 h.

**Statistics** Data of growth promotion and yield were analyzed by the analysis of variance (ANOVA). The data of repeated trials were tested for homogeneity of results. Isolates which promoted growth were selected for statistical analyses. The data were analyzed by ANOVA in the split-plot design by taking fungal isolates as main plot factors and varieties as sub-plot factors. The data of the field experiment were also analyzed as above with seven fungal isolates and two varieties as main plot and sub-plot factors, respectively. The fungal treatment means and varietal means were separated by employing Duncan's multiple range test (DMRT, $P=0.05$) and Fisher's least significant difference test (LSD, $P=0.05$, 0.01), respectively.

## Results

**Greenhouse assay** Of the 21 isolates, nine promoted ($P=0.05$) the growth of soybean plants of both varieties, while others failed. Compared with the untreated control, the increases in length and biomass of plants caused by the nine isolates ranged from 22% to 66% and from 21% to 42%, respectively, for var. 1, and 21% to 42%, respectively, for var. 2. Among the nine isolates, GS7-4, GS8-2, and GS8-3 were most effective in increasing the plant length and plant biomass of var. 1 compared with the untreated control. On the other hand, isolates GS7-4, GS8-3, GS10-1, GS10-2, and GT2-1 were most effective in increasing the plant length, and all of these isolates except GS7-4, increased the plant biomass of var. 2 (Fig. 1).

All isolates that enhanced the length and biomass of plants also caused significant ($P=0.05$) increases in the number of pods, pod dry biomass, the number of seeds and seed dry biomass of both varieties. In the case of var. 1, GS8-3 induced a consistent increase of all yield components. GS7-4 and GS8-2 increased yield ($P=0.05$), but the increase was not consistent. However, GU23-3, GT2-1, and GS6-2 enhanced the yield components, particularly, the number of seeds and seed dry biomass, compared to their low ability to enhance langth and biomass of plants. The increases in the number of seeds and seed dry biomass by different isolates ranged from 21% to 60% and from 15% to 39%, respectively. In case of var. 2, GS10-2 and GT2-1

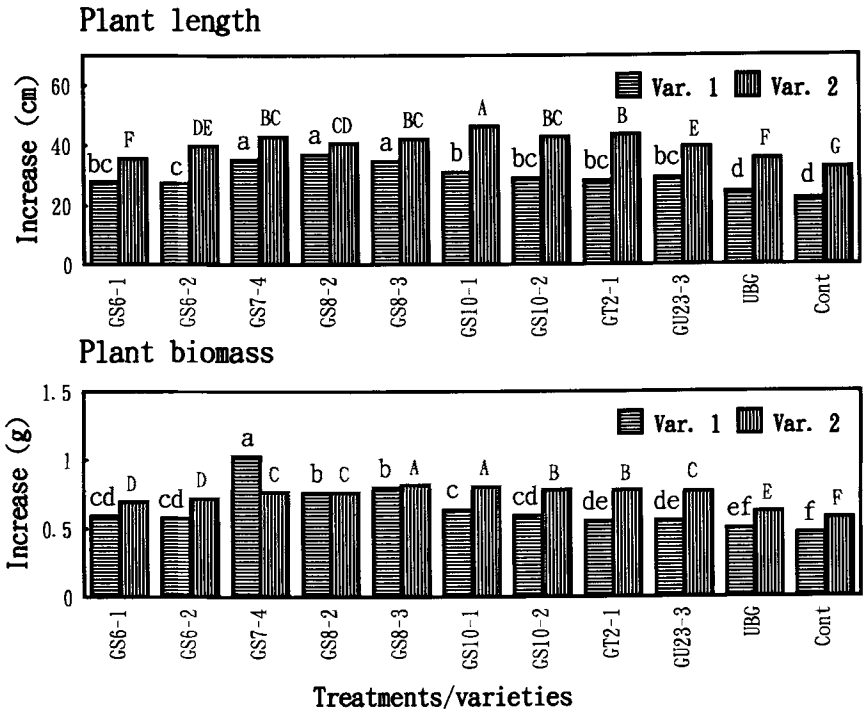


Fig. 1. Effect of soil amendment with barley grain inocula (1%, w/w) of plant growth promoting fungal isolates on the length and biomass of soybean varieties (Var. 1 and Var. 2) grown for nine weeks in the greenhouse. Bars carrying the same letter are not significantly different (DMRT, P=0.05). LSDs for comparing means of length of two varieties are 2.2 cm and 2.3 cm at P=0.05 and P=0.01, respectively. LSDs for comparing means of biomass of two varieties are 0.053 g and 0.073 g at P=0.05 and P=0.01, respectively.

followed by GS8-3, GU23-3, and GS6-2 induced a consistent increase in yield components. The increases in the seed number and seed dry biomass by the nine select isolates ranged from 23% to 68% and from 28% to 59%, respectively (Table 1).

Soil amendment with uninfested barley grain did not show a significant increase in plant length and plant biomass of var. 1. However, the increase in case of var. 2

Table 1. Yield components[1] of soybean varieties (V1 and V2) grown in soil unamended or amended with barley grain inocula (1%, w/w) of plant growth promoting fungal (PGPF) isolates under greenhouse conditions.

| PGPF Isolates | Number of pods | | Pod dry biomass (g/plant) | | Number of seeds | | Seed dry biomass (g/plant) | |
|---|---|---|---|---|---|---|---|---|
| | V1 | V2 | V1 | V2 | V1 | V2 | V1 | V2 |
| GS6-1 | 5.8 b[2] | 3.9 c | 2.161 c | 1.435 c | 4.6 d | 4.7 e | 1.258 a-c | 1.037 b |
| GS6-2 | 5.6 b | 4.5 ab | 1.994 c | 1.833 b | 4.6 d | 5.9 bc | 1.330 a | 1.238 a |
| GS7-4 | 6.9 a | 4.6 ab | 2.880 a | 1.803 b | 5.6 ab | 5.6 c | 1.293 a-c | 1.101 b |
| GS8-2 | 5.5 b | 4.4 ab | 2.104 c | 1.473 c | 4.8 cd | 5.0 de | 1.194 b-d | 1.039 b |
| GS8-3 | 6.3 a | 4.4 ab | 2.598 b | 1.437 c | 6.1 a | 5.9 bc | 1.357 a | 1.040 b |
| GS10-1 | 5.5 b | 4.7 a | 2.003 c | 1.988 a | 4.8 cd | 5.2 d | 1.163 cd | 1.100 b |
| GS10-2 | 5.5 b | 4.3 b | 2.074 c | 1.787 b | 4.6 d | 6.3 ab | 1.117 d | 1.278 a |
| GT 2-1 | 5.5 b | 4.9 c | 2.022 c | 1.747 b | 5.6 ab | 6.4 a | 1.322 ab | 1.293 a |
| GU23-3 | 5.5 b | 4.0 c | 2.000 c | 1.430 c | 5.3 bc | 6.0 a-c | 1.252 a-c | 1.242 a |
| UBG[3] | 5.5 b | 3.9 c | 1.985 c | 1.435 c | 4.6 d | 5.0 de | 1.083 e | 1.037 b |
| Control | 4.6 c | 2.9 d | 1.693 d | 1.100 d | 3.8 e | 3.8 f | 0.970 e | 0.810 c |
| LSD (P=0.05) | 0.5 | | 0.214 | | 0.3 | | 0.120 | |
| (P=0.01) | 0.7 | | 0.291 | | 0.5 | | 0.163 | |

[1] Mean of two trials each with three replicates.
[2] Values followed by the same letter are not significantly different (DMRT, P=0.05).
[3] UBG=Soil amended with 1% (w/w) autoclaved, uninfested barley grains.

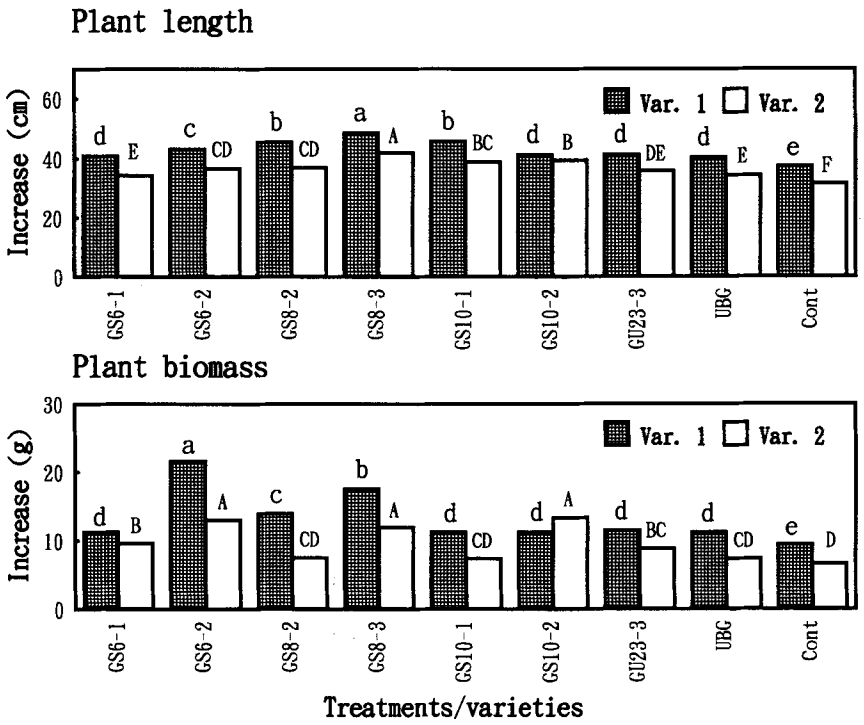


Fig. 2. Effect of soil amendment with barley grain inocula (20 g/row) of plant growth promoting fungal isolates on the length and biomass of soybean varieties (Var. 1 and Var. 2) grown for nine weeks in the field. Bars carrying the same letter are not significantly different (DMRT, P=0.05). LSDs for comparing means of length of two varieties are 1.3 cm and 1.8 cm at P=0.05 and P=0.01, respectively. LSDs for comparing means of biomass of two varieties are 0.93 g and 1.28 g at P=0.05 and P=0.01, respectively.

was significant (P=0.05) compared to the untreated control (Fig. 1). With respect to yield components, plants grown in soil amended with uninfested barley grains produced significant (P=0.05) increases in the number of pods, pod dry biomass, seed number and seed dry biomass over those of plants grown in unamended soil. However, such an increase in yield components was less than that caused by certain isolates (Table 1).

The significant (P=0.01) interaction found between isolate treatments and varieties indicates that the overall

Table 2. Yield components[1] of soybean varieties (V1 and V2) grown in soil unamended or amended with barley grain inocula (20 g/row of 10 m length) of plant growth promoting fungal (PGPF) isolates under field conditions.

| PGPF Isolates | Number of pods | | Pod dry biomass (g/plant) | | Number of seeds | | Seed dry biomass (g/plant) | |
|---|---|---|---|---|---|---|---|---|
| | V1 | V2 | V1 | V2 | V1 | V2 | V1 | V2 |
| GS6-1 | 47.5 e[2] | 42.7 c | 45.9 b | 43.2 b | 99.6 c | 86.6 d | 25.0 ab | 19.2 c-e |
| GS6-2 | 51.0 d | 42.7 c | 45.9 b | 43.5 b | 99.0 c | 109.3 b | 23.7 b | 20.9 c |
| GS8-2 | 58.1 b | 38.2 cd | 45.9 b | 40.2 b | 99.0 c | 86.6 d | 24.9 ab | 17.2 ef |
| GS8-3 | 64.5 a | 56.3 a | 58.1 a | 46.6 a | 116.9 a | 92.3 c | 26.3 a | 24.9 b |
| GS10-1 | 50.9 d | 37.9 d | 45.9 b | 39.9 c | 97.9 c | 91.0 c | 23.9 b | 18.6 de |
| GS10-2 | 50.9 d | 37.9 d | 45.9 b | 39.9 c | 97.9 c | 90.8 c | 23.6 b | 19.5 cd |
| GU23-3 | 55.3 c | 45.7 b | 46.9 b | 45.0 ab | 109.1 b | 111.7 a | 25.8 a | 27.3 a |
| UBG[3] | 50.8 d | 38.0 d | 45.8 b | 39.9 c | 98.0 c | 85.9 d | 23.6 b | 18.0 d-f |
| Control | 44.5 f | 30.1 e | 39.5 c | 32.0 d | 83.4 d | 76.8 e | 21.1 c | 16.3 f |
| LSD (P=0.05) | 1.9 | | 2.6 | | 2.5 | | 2.4 | |
| (P=0.01) | 2.6 | | 3.5 | | 3.4 | | 3.3 | |

[1] Average of two trials each with three replicates.
[2] Values followed by the same letter are not significantly different (DMRT, P=0.05).
[3] UBG=Soil amended with autoclaved, uninfested barley grains (20 g/row).

growth promotion effect of the fungal isolates depends not only on their inherent ability but also on the response of varieties to fungal treatment (Table 1).

**Field trial** Compared with the untreated control, the increase in length and biomass of plants caused by the seven selected isolates ranged from 9% to 30% and from 19% to 128%, respectively, for var. 1, and from 8% to 32% and from 12% to 103%, respectively, for var. 2. Isolates GS6-2, GS8-2, and GS8-3 were effective ($P=0.05$) in enhancing the length and biomass of plants of var. 1 compared with the untreated control. GS10-1 was more effective in increasing the length than the biomass. For var. 2, the most effective isolates in enhancing the plant length were GS8-3, GS10-1, and GS10-2, while, GS8-3 and GS10-2 along with GS6-2 were most effective for plant biomass increase, and GS10-1 was less effective (Fig. 2).

Regarding the yield components, in the case of var. 1, GS8-3 and GU23-3 showed a consistent and significant ($P=0.05$) increases in pod number, pod dry biomass, seed number and seed dry biomass. The other isolates, although causing significant increases over those of untreated control, were less effective. The increases in seed number and seed dry biomass were 17-40% and 11-24%, respectively over untreated control. In var. 2, GU23-3 and GS8-3 induced a consistent and significant ($P=0.05$) increase in yield. Soil amendment with the selected fungal isolates induced increases in seed numbers of 11% to 24% and in seed dry biomass of 14% to 67% (Table 2).

Plants of var. 1 grown in soil amended with autoclaved, uninfested barley grains also showed increases ($P=0.05$) in plant length and plant biomass compared to those grown in unamended soil. In the case of var. 2, an increase in length and biomass of plants was noted but it was not significant (Fig. 2). The yield components of plants grown in uninfested barley grain-amended soil showed a similar tendency to those of greenhouse-grown plants (Table 2).

As in the greenhouse assay, ANOVA revealed significant ($P=0.01$) interactions between fungal treatments and varieties.

None of the fungal isolates tested caused any deleterious effect on roots and shoots. However, certain isolates cased non-significant decreases in length and biomass of plants compared with the untreated control. Similar observations were made regarding the effect of such isolates on yield.

## Discussion

Among 21 rhizosphere fungal isolates tested for plant growth promotion, only a few isolates induced consistent increases in growth and yield in the greenhouse and field. These isolates promoted growth significantly in both greenhouse and field and are referred to as 'plant growth promoting fungi' (PGPF). Most of the isolates tested in the present study were good plant growth promoters of spring wheat (Shivanna et al., 1993) and corn (*Zea mays* L.), while only certain isolates promoted the growth of cucumber (Shivanna et al., unpublished). This suggests that zoysiagrass rhizosphere isolates might associate well with roots of monocotyledonous crops like wheat and corn. This is further supported by the fact that more of these isolates colonize roots of wheat (Shivanna et al., unpublished) than roots of cucumber (Meera et al., 1995). A similar phenomenon has been observed by Dewan and Sivasithamparam (1989a). They showed that the sterile red fungus from rhizospheres of rye-grass (*Lolium rigidum* L.) and wheat belonging to the group Basidiomycetes enhanced the growth of cereal crops better than that of non-cereal crops (Dewan and Sivasithamparam, 1989b).

In the present study, certain isolates induced higher plant growth promotion in the greenhouse than in the field. This could be due to the weaker competition by the local microbes in the greenhouse soil compared with the large number of microbes in the field soil. The growth promotion activity of the PGPF isolates might depend on their competitive saprophytic ability (Shivanna et al., unpublished) and their ability to endure antagonism by the local antagonists of the soil. In the greenhouse soil, plant growth was higher in PGPF amended soil than in the unamended soil. Analyses of samples of soil used for greenhouse and field experiments show that the C and P contents are higher in the field soil than the greenhouse soil. Further, results of the greenhouse experiment show that certain isolates perform better in the nutrient-depleted soil of the greenhouse than in the nutrient-rich field soil. These results corroborate the findings of Balis (1970), who showed a good response of wheat plants to *Phialophora graminicola* (Deacon) Walker in nutrient-depleted soil. Deacon (1973) also demonstrated this in a *Festuca-Agrostis* mixture.

Association of PGPF isolates with roots might help plants to derive mineral nutrients in the readily available form as has been shown in case of wheat (Shivanna et al., 1994). This might be closely related to the root colonization ability of PGPF isolates. Dewan and Sivasithamparam (1989b, 1990) demonstrated that the sterile red fungus invaded the inner root regions which helped plants in deriving nutrients from the soil and in protecting roots against pathogens. Cowan (1979) considers the possibility that some growth-promoting isolates of *P. graminicola* act to increase the mineral uptake by plants in the same way as do mycorrhizal fungi. A similar possible mechanism of growth promotion has also been suggested by Barber and Lynch (1977) and Brown (1974).

Plant growth promotion could also be due to the mineralization of barley grains by PGPF isolates during colonization. The sterile isolates have been shown to colonize barley grains and increase their content of ammonium-nitrogen, which is easily absorbed by roots resulting in the increased plant growth (Shivanna et al., 1994).

In both greenhouse and field, certain PGPF isolates showed variety-specific responses suggesting their inherent ability to associate with plants of particular varieties. Howie and Echandi (1983) suggested that the type of potato cultivar and the type of soil, besides the strain

of rhizobacteria, influence the growth and yield. He stated that the variation in the plant growth response of different varieties, irrespective of their association with PGPR isolates, could be due to the varietal make-up. Dolan et al. (1986) also observed similar species-specific growth-promotion responses of different species of *Persea* to non-pathogenic strains of *Phytophthora parasitica* Dastur.

The present study suggests that the plant growth promotion by PGPF isolates depends on the type of cultivar and the soil nutrient conditions besides the inherent ability of isolates.

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