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***Original Research Article***

# Biopriming of *Salvia officinalis* Seed with Growth Promoting Rhizobacteria Affects Invigoration and Germination Indices

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## ABSTRACT

**Plant growth promoting rhizobacteria (PGPRs) are a group of bacteria that can actively colonize plant roots and can modulate plant growth. The present study was aimed to investigate the effects of three PGPRs strains designated as *Pseudomonas fluorescens* (PF-23) and *P. putida* (PP-41 and PP-159) on seed germination features including germination percentage (GP), mean time germination (MTG), germination rate (GR), root and shoot length and seedling vigor index (VI) of *Salvia officinalis* L. The bacterial suspension ($10^9$ CFU/ml) was used to inoculate the seeds under aseptic conditions. Results revealed that seed treatment of *S. officinalis* with rhizobacteria including PF-23, PP-41and PP-159 affects differently germination parameters. The maximum (78.5%) and minimum (16.75%) final GP were recorded in PP-41 and PF-23 treatment, respectively. Also, the highest GR, root and shoot length, seedling VI and the lowest MTG were recorded in seeds treated with PP-41, a strain with ability to produce moderate auxin, when compared to the other treatments. It is concluded that net effect of plant–rhizobacteria interactions on seed germination behaviors could be positive, neutral or negative. On the other hand, different strains of rhizobacteria had variable effects *i.e.*, positive, negative and inconsequential effects of PGPRs application were observed on seed germination, root elongation and subsequently seedling VI. As a conclusion, the role of biopriming with PGPRs on germination characteristics and seedling growth varied with bacteria strains.**

**Key Words: *Salvia officinalis*, PGPRs, Biopriming, Invigoration**

**Abbreviations: PGPRs: Plant growth promoting rhizobacteria; GP: Germination percentage; MTG: Mean time germination; GR: Germination rate; VI: Vigor index; PF: *Pseudomonas fluorescens*; PP: *Pseudomonas putida***

## INTRODUCTION

In recent years, an upsurge of interest in the use of natural substances as phytomedicines has resulted in a more thorough investigation of plant resources. Salvia is an important genus of the family Lamiaceae that includes more than 700 species which are spread throughout the world (Ewans, 1996). Most of salvia species are, commonly, utilized for their essential oils in the foods, medicines and perfumery industries (Goren *et al.*, 2006; Ozcan *et al.*, 2003).

One of the main problems that prevent sustainable use of medicinal plants, native to the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions (Gupta, 2003) or when cultivation is attempted.

A simultaneous development of easy-to employ means of *ex situ* propagation of the species concerned would encourage their cultivation, thereby considerably easing the pressure on natural habitats. The common means of regeneration and propagation of medicinal plants include seed-based, clonal and micropropagation methods. Seed-based multiplication is the most effective, realistic and convenient means for most species. Moreover, seed germination simultaneous of medicinal and aromatic species can provide more uniformity of germination, more uniform seedlings emergence and subsequently more uniform plants at other stages like flowering, a critical time to achieve more bioactive secondary metabolites.

In recent years, a lot of studies have been done on invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of many vegetables and some field crops (Basra *et al.* 2003). Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops (Halmer, 2003; Taylor and Harman, 1990). However, a limited number of studies have been undertaken regarding the plant growth promoting rhizobacteria (PGPRs) and

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medicinal plant interactions. Seed priming may be used as an important tool to improve seed performance and stand establishment in the field (Nascimento and Pereira, 2007).

Interest in study of PGPR has recently increased, due to the potential for improving growth and yield of various crops. PGPRs are naturally occurring soil bacteria that aggressively colonize the rhizosphere, rhizoplane and improve plant growth when artificially introduced onto seeds, seed pieces, roots, or into soil.

Jahanian *et al*. (2012) studied the seed inoculation of artichoke (*Cynara scolymus*) with different plant growth promoting rhizobacteria (*Azotobacter*, *Azospirilium*, *Pseudomonas putida* 41, *Pseudomonas putida* 168) on germination and plant early growth characteristics. Based on their experiments they concluded that the highest germination percentage, mean time of germination, number of normal plants, radicle and shoot weight, shoot length and vigority were observed in plants treated with PP168. They also reported that either sole or integrated application of phosphorus solublizing bacteria along with nitrogen fixing ones led to significant increase in radicle and shoot length, shoot weight, coefficient of velocity of germination, seedling vigor index, and to significant decrease in mean germination time compared to control. The test rhizobacterium P-35 with multiple PGP activities like IAA, ammonia, HCN (hydrogen cyanide), catalase etc. was subjected to seed germination test for *Withania somnifera* plants. The results established a significant enhancement in seed germination as well as root and shoot growth of this valuable medicinal plant (Rathaur *et al.,* 2012). Although PGPRs have been continuously used to enhance the seed germination and overall yield of many crops in different agro-ecosystems, there is a lack of available reports on medicinal and aromatic species seed germination and vigor index. However, the objective of this study was conducted to investigate the efficiency of PGPRs, *Pseudomonas Fluorescens* and *Putida* strains, on sage (*Salvia officinalis* L.) seed germination and seedling vigor index.

## MATERIALS AND METHODS

### *PGPR strains*

Three strains of *Pseudomonas fluorescens* (PF-23) and *Putida* (PP-41 and PP-159) were obtained from the department of microbiology, soil and water research Institute, Karaj, Iran. PGPR strains with different multiple growth promoting characteristics and various ability in auxin (IAA) production were used for laboratory experiment (Table 1 and 2). To prepare inoculums, a single colony of each PGPR strain was transferred to 100 ml flasks containing 25 ml of TSB (tryptone soybean broth) and grown aerobically in flasks on a rotating shaker (120 rpm) for 72 h at 28°C. The bacterial suspension was then diluted in sterile distilled water to achieve a final concentration of $10^9$ CFU/ml. The prepared suspensions were used to inoculate *S. officinalis* seeds under aseptic conditions.

### *Seed preparation and inoculation*

Seeds of *Salvia officinalis* L. plant were collected from their natural habitats in Hesar [longitude (E): 49°17′23.9″, latitude (N): 33°59′6″, altitude (m): 2361, collection date: 2012.July.30], Markazi province, Iran, for testing the effects of reference PGPRs on germination indices including germination percentage (GP), mean time germination (MTG), and germination rate (GR) and seedling vigor index (VI). At first, the seeds were surface sterilized by soaking in 1% NaOCl for 10 min and subsequently rinsed thoroughly with sterilized water prior to applying the employed treatments. Twenty seeds of *S. officinalis* were selected and placed in each Petri dish and treated with solutions containing the above mentioned bacterial media. In control, only distilled water was used. In treatments, 8 ml of solution containing different bacterial media was added. Four dishes were used for each treatment and control. After each treatment, seeds were transferred to germinator with 16/8 h photoperiod, constant temperature of 22 ºC and relative humidity of 70%. The seeds were considered to be germinating at the moment of radicle emergence. The number of germinated seeds was recorded daily and the final percentage of germination was measured after two weeks.

***Germination analysis***

The final germination percentage was calculated based on total number of germinated seeds at the end of tenth day. The measurements were done according to International Rules for Seed Testing (ISTA, 1985). Germination indices as well as vigor index were calculated using the following equations (Alvarado *et al.* 1987, Ruan *et al.* 2002, Ellis and Roberts 1981):

Germination percentage (GP %) = (Gf/N) 100, where Gf is the total number of germinated seeds at the end of experiment and N is the total number of seed used in the test.

Mean Time Germination (MTG) was calculated according to following equation:
MTG = Σ NiDi/N, Where Ni is number of germinated seeds till ith day and Di is number of days from start of experiment till $i^{th}$ counting and N is total germinated seeds.

Germination rate (GR) = Σ Ni/ΣTi Ni, where Ni is the number of newly germinated seeds at time Ti. Vigor index (VI) = SDW G%, where SDW is seedling length at the end of test and GP% is the final germination percentage.

***Statistical Analysis***

Data were processed by the analysis of variance (ANOVA) on the basis of completely randomized design (CRD) with 4 replications. The data were analyzed using computer SAS software (version 9.1; CoHort Software), and the means were compared by Duncan's multiple range test ($P < 0.05$).

**Table 1.** Multiple plant growth promoting characteristics of *Pseudomonas putida* strains (PP- 41 and 159) and *Pseudomonas fluorescens* (PF-23).

| PGPR strains | PGPR characteristics | | | | | Ecological site of strains isolation (rhizosphere) |
|---|---|---|---|---|---|---|
| | Phosphorus solubility | | Siderophore Production (halo/colony) | HCN production | ACC deaminase activity | |
| | µg.ml$^{-1}$ | halo/colony | | | | |
| PP-41 | 228.20 | 1.28 | 1.98 | - | - | Wheat |
| PP-159 | 374.86 | 3.11 | 2.21 | low | + | Wheat |
| PF-23 | 338.30 | 1.52 | 2.73 | very low | - | Canola |

PGPR: Plant Growth Promoting Rhizobacteria, *Irrigated farming variety (Hydrophyl), **Dry land farming variety (Xerophyl), HCN: Hydrogen Cyanide, ACC: 1-aminocyclopropane-1-carboxylic acid.

**Table 2.** Auxin production (IAA) of PGPR strains under different concentration of Tryptophan (mg.L$^{-1}$).

| PGPR | Tryptophan* (mg.L$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 50 | 100 | 200 |
| PP-41 | 0.17 | 6.45 | 8.08 | 21 |
| PP-159 | 0.22 | 10.17 | 25.19 | 39.71 |
| PF-23 | 0 | 1.09 | 10.45 | 63.7 |

*Tryptophan is a key precursor in IAA biosynthesis. IAA production of PGPR strains were increased with increasing the concentration of tryptophan from 0 to 200 mg.L$^{-1}$

## RESULTS AND DISCUSSION

Analysis of variation revealed that the effects of bio-priming with inoculation of PGPRs had significant ($P$ < 0.01) influence on all measured parameters including GP, MTG, GR, seedling length and subsequently VI (Table 3). When treatments were compared to each other, it was observed that the effects of PGPRs on seed germination characteristics and early seedling growth varied with bacteria strains (Table 4). Results of comparing different PGPRs showed that biopriming had positive, negative and inconsequential effects on germination and growth parameters. For instance, GP was higher (78.50%) in bio-priming with *Pseudomonas putida* (PP-41) followed by PP-159 (57.75%), control (41.25%) and *Pseudomonas fluorescens* (PF-23) strain (16.75%). However, the highest MTG (9.75 days) and the lowest of that (4.25 days) were recorded for seeds treated with PF-23 and PP-41, respectively. GR changes trend in seeds under different PGPRs inoculation and in control untreated seeds were similar to GP character. On the other hand, the maximum (1.05 seeds/day) and the minimum (0.17 seeds/day) GR were calculated in PP-41 and in PF-23 treatments, respectively (table 4). Moreover, the highest root (8.45 cm) and shoot (4.20 cm) length and subsequently VI (992.12) obtained for seeds primed with PP-41 strain, while the lowest of VI observed in PF-23 treated seeds (Fig. 1). Seeds, *i.e.*, primed with both PP strains (41 and 159) were significantly superior in root, shoot and seedling lenght, compared with other treatments. In our current study, different bacterial treatments had diverse effects (stimulative or inhibitory) on seed germination of *S. officinalis* L. For example, treatment with PP-41 and PP-159 stimulated seed germination and seedling growth, while opposite results were obtained for PF-23 treatment. In general, it was obvious that biopriming with both *Pseudomonas putida* strains increased the GP, GR, root and shoot length and VI compared to the control; however, the most ineffective strain was *Pseudomonas fluorescens* strain 23. The highest GP value along with the lowest MTG, which show more uniformity of germination and emergence, was observed in seeds treated with PP-41 strain.

**Table 3.** Analysis of variances (ANOVA) of measured parameters in *S. officinalis* under different treatments of PGPRs inoculation.

| Source of Variation (S.O.V) | Degree of freedom (df) | Mean of Square | | | | | |
|---|---|---|---|---|---|---|---|
| | | Germination percentage | Mean germination time | Germination rate | Root length | Shoot length | Vigor index |
| Treatment | 3 | 2728.22$^{**}$ | 20.729$^{**}$ | 0.5646$^{**}$ | 29.99$^{**}$ | 4.385$^{**}$ | 651109.4$^{**}$ |
| Error | 12 | 16.604 | 19.37 | 0.0029 | 0.2512 | 0.1068 | 2332.29 |
| CV (%) | | 8.39 | 19.03 | 7.98 | 9.5 | 11.1 | 10.2 |

$^{**}$ Significant statistically at 1%.

**Table 4.** Seed germination behavior and seedling vigor index of *Salvia officinalis* under biopriming with *Pseudomonas putida* (PP- 41 and 159) and *P. fluorescens* (PF-23) strain.

| PGPR strain | Germination indices | | | | | |
|---|---|---|---|---|---|---|
| | Germination percentage (%) | Mean germination time (day) | Germination rate (seed/day) | Root length (cm) | Shoot length (cm) | Vigor index |
| Control | 41.25$^{c}$ | 7.75$^{ab}$ | 0.66$^{c}$ | 3.92$^{c}$ | 2.32$^{c}$ | 257.03$^{c}$ |
| PP-41 | 78.50$^{a}$ | 4.25$^{c}$ | 1.05$^{a}$ | 8.45$^{a}$ | 4.20$^{a}$ | 992.13$^{a}$ |
| PP-159 | 57.75$^{b}$ | 7.5$^{b}$ | 0.83$^{b}$ | 6.47$^{b}$ | 3.37$^{b}$ | 570.40$^{b}$ |
| PP-23 | 16.75$^{d}$ | 9.75$^{a}$ | 0.17$^{d}$ | 2.25$^{d}$ | 1.87$^{c}$ | 69.63$^{d}$ |

In each column, values followed by different letters differ significantly ($P$ < 0.01) according to Duncan's multiple range test.

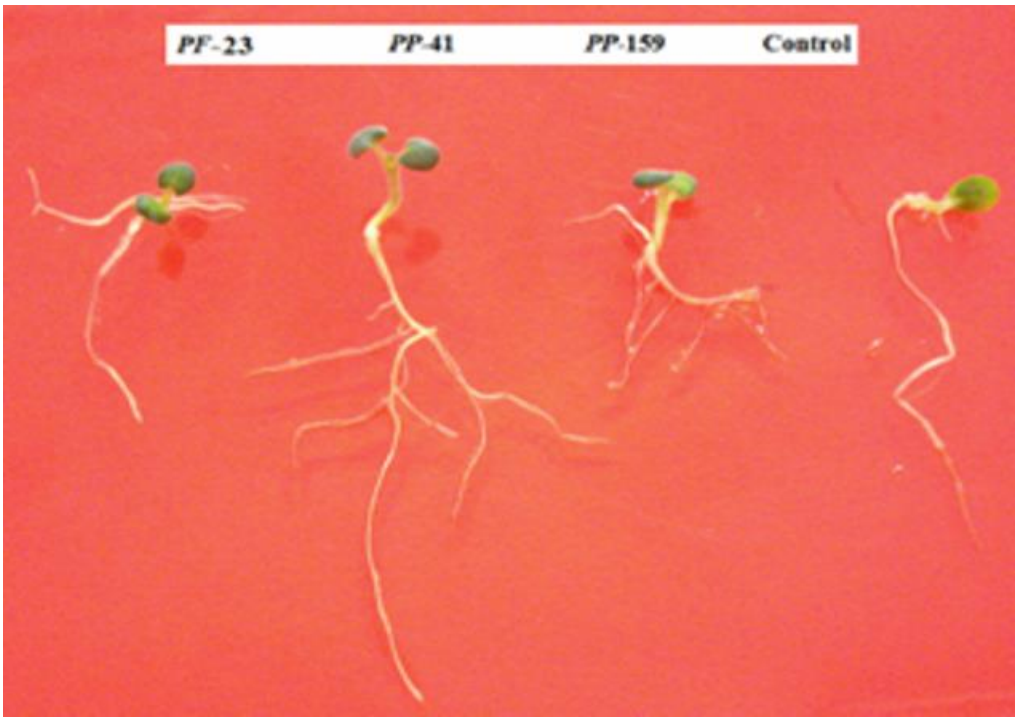


**Fig. 1**. *Pseudomonas putida* (PP- 41, 108 and 159 strains) and *P. fluorescens* (PF-23 strain) seed inoculation effects on root morphology and seedling vigor index of *Salvia officinalis* L.

It has been reported that PGPRs are able to improve plant growth by increasing the rate of seed germination and seedling emergence (Shaukat *et al.*, 2006). The role of PGPRs on *Hyoscyamus niger* seedling growth was evaluated by Ghorbanpour and Hatami (2013). Based on their experiments the inoculation of *H. niger* seedling radicles with different twenty plant growth promoting rhizobacteria strains belonging to *Pseudomonas putida* (PP) and *P. fluorescens* (PF) on VI under two various conditions, *in vitro* (with agar media) and tube (sand culture) assays, indicated that the most efficient strains on VI were those (PP-168 and PF-187) that produce optimum auxin. They also reported that different strains of rhizobacteria had variable effects (both negative and positive) on VI in two various tested assays. They also reported that PF-187 strain increased root and shoot elongation by 73% and 51% compared with un-inoculated control, respectively. Moreover, two strains of PP (strains 4 and 11) had negative effects on vigor index, when compared with the control. Under both assays conditions PF-187 and PP-168 strains were the most effective strains for early seedling development (Ghorbanpour and Hatami, 2013). Fluorescent pseudomonads including *putida* and *fluorescens* have substantial effects on plant growing under various conditions particularly via auxin secretion. However, production of this phytohormone at the amounts higher than that is needed for plant produces additional levels of ACC (1-aminocyclopropane 1-carboxylic acid), the immediate precursor of ethylene production, which significantly inhibits root elongation and decreases VI and plant growth (Fig. 2) (Glick *et al.*, 1998).

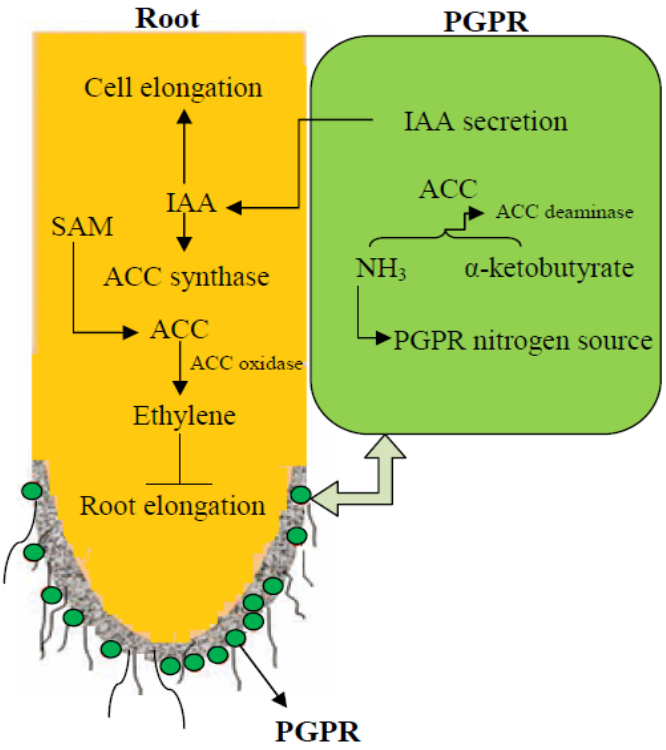


**Fig. 2.** Schematic model of PGPRs effects on root grwoth. IAA; Indole acetic acid, ACC; 1-aminocyclopropane 1-carboxylic acid, SAM; S-adenosylmethionine.

Seeds of various plants bacterized with a mixture of PGPR and rhizobia before planting have resulted in enhanced growth and efficiency of induced disease resistance (Zehnder *et al.,* 2001). It is well known that growth promotion in response to PGPRs inoculation may involve various mechanisms of action. Most PGPR strains may work through multiple mechanisms, which accounts for the observed beneficial effects on plant growth. Many researchers are of the view that a very important mechanism of direct growth promotion by PGPRs may be the production of plant growth regulators like indole acetic acid (IAA) (Mishra *et al.,* 2010), gibberellic acid (Narula *et al.,* 2006), cytokinins (Castro *et al.,* 2008) and ethylene (Saleem *et al.,* 2007). For example, Nelson (2004) noted that PGPRs were able to exert a beneficial effect upon plant growth such as increasing the germination rate. Thus, the reasons by which the PGPRs enhance seed germination and subsequently seedling vigority may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as amylase, which have brought an increase in availability of starch assimilation. Besides, significant increase in seedling vigor would have occurred by better synthesis of auxins (Bharathi, 2004). A commercial soil amendment containing a mixture of four PGPR *(Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens and Bacillus megaterium)* was evaluated for impact on germination and initial growth of *Catharanthus roseus* (Lenin and Jayanthi, 2012). They reported that root inoculation of PGPR strains significantly increased GR and VI. Also, the highest percentage of seed germination and VI of 87.10% and 1988.1 was recorded for consortium treated *C. roseus* followed by single inoculant treatment. Similarly, five bacterial strains (TR1 to TR5) from root nodules of fenugreek (*Trigonella foenum-graecum*) were tested for their plant growth promotory traits by Harish Kumar *et al.* (2011). They concluded that maximum increments in VI, nodule number and root and shoot biomass were recorded with seed inoculated with consortium (TR1+TR2) followed by single inoculation as compared to control. Sing *et al.* (2011) treated two *Acacia senegal* genotypes seeds with *Bacillus licheniformis* or *Sinorhizobium saheli*, either inoculated individually or as coinoculants, and recorded positive effect on phenotypic traits of germination. There is also report concerning the inoculation of *Ambrosia artemisiifolia* with *P. fluorescens*, resulting in inhibition of seed germination (Vrbnicanin *et al.,* 2011). Our results are in consistent

with the findings of Zdor *et al.* (2005) and Jaleel *et al.* (2007), which suggested that *P. fluorescens* was sometimes classified as deleterious rhizobacteria (DRB) (Zdor *et al.*, 2005) and sometimes as PGPR (Jaleel *et al.*, 2007) that depend on conditions in which bacterial cultures develop. From the results it can be concluded that the function of biopriming with PGPRs on germination characteristics and seedling early growth varied with bacteria strains based on multiple growth promoting characteristics.

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