Australian Journal of Crop Science AJCS
AJCS 4(7):564-570 (2010) ISSN:1835-2707

Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas fluorescens* for improvement of seed invigoration and seedling growth

A. Moeinzadeh[1*], F. Sharif-Zadeh[1], M. Ahmadzadeh[2], F. Heidari Tajabadi[2]

[1]Department of Agronomy and Plant Breeding, Faculty of Agricultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[2]Department of Plant Protection, Faculty of Agricultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

***Corresponding author: asma_k_61@yahoo.com**

Abstract

Biopriming treatment is potentially able to promote rapid and more uniform seed germination and plants growth associated with bacterial coatings. In this study, we report application of some effective biological agents on sunflower seed and their impact on seedling. We investigated the effects of 30 strains of *Pseudomonas fluorescens* on improving sunflower seed germination and promotion of seedling growth. After selection of efficient strains, efficacy of biopriming seed treatment was compared with seed inoculation and priming treatments. Two strains, including UTPf76 and UTPf86, were selected for next experiments because they enhanced seed factors such as germination index, germination percentage, germination rate and vigor index and also seedling growth indices including root length, shoot height, dry and wet weight of seedlings and numbers of lateral roots. In biopriming, the selected strains were applied to the seed during osmopriming with NaCl. Biopriming was significantly improved by the capability of these strains and the highest amount (P= 0.01) of shoot height (28.2 cm), root length (35.9 cm) and seedling weight (8.9gr) reached, in comparison with other treatments and the control. As a conclusion, biopriming with *Pseudomonas fluorescens* UTPf76 and UTPf86 have provided very well establishment and adherence of bacteria to the seed, before planting, and thus is suggested as a proper treatment for enhancement of seed indices and improvement of seedling growth.

Keywords: Biopriming, *Pseudomonas fluorescens*, Seed invigoration, Seedling growth improvement, Sunflower
Abbreviations: CFU _Colony forming unit; MC_ Methyl cellulose; PGPR_ Plant growth promoting rhizobacteria; UTPf_ University of Tehran *Pseudomonas fluorescens*

Introduction

Sunflower (*Helianthus annuus* L.) is a high yielding oilseed crop, but under scarce conditions, the yield is very lower than its real potential. Among the factors responsible for the low yield, imbalance use of fertilizers, improper plant protection, poor growth and sub optimum plant population are rather important. Suboptimum plant population generally results from poor and erratic germination. 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). In priming, seeds are exposed to restricted water availability under controlled conditions which allows some of the physiological processes of germination to occur and then, before germination is completed, the seeds are usually re-dried for short term storage before sowing (Halmer, 2000; Halmer, 2003; Taylor and Harman, 1990). At least three technologies exist to achieve priming including hydropriming, osmopriming and solid-matrix priming (Halmer, 2003; Ashraf and Foolad, 2005). Historically, the most widely used world-wide method is osmopriming where seeds are placed in aerated solutions containing mannitol, inorganic salts or polyethylene glycol of low water potential. Previous studies revealed the beneficial effects on seed germination and seedling emergence on priming lettuce seeds occurred following a high temperature (35°C) and/or incorporating with plant growth regulators (Korkmaz, 2006). Seed priming may be used as an important tool to improve seed performance and stand establishment in the field, especially during the summer (Nascimento and Pereira, 2007). Interest in study of PGPR has recenly increased, due to the potential for improving growth and yield of various crops. Lifshitz et al. (1987) studied the growth promoting activity of *pseudomonas putida*, strain GR 12-2, in a gnobiotic growth pouch assay, reporting that inoculation of rapeseed with GR 12-2 significantly increased the root length, shoot height and phosphorus uptake, compared with an un-inoculated control. Significant yield increases in many other crops have also been reported in response to inoculation with PGPR (Iswandi et al., 1987; Javed et al., 1996; Khalid et al., 1997; Zahir et al., 1997, 1998a, 1998b). To get maximum benefit of inoculation, the selection of the most effective PGPR is a prerequisite. However, there has been no standard approach for selection of effective PGPR. The use of rhizobacterial strains directly in the field without screening is a highly laborious procedure that demands on effective screening of the rhozobacteria before taking them to the field. Inoculation of seeds with biological agents in combination with priming has, in several cases, been reported to enhance and stabilize the efficacy of biological agents (Callan et al., 1990, 1991; Harman et al., 1989; Warren and Bennett, 1999). Bio-osmopriming

Table 1. 30 strains of bacteria which were used in this study and their precise specifications.

Strain	Species	Host plant	Isolating location
UTPF2	*Pseudomonas fluorescens*	Turnip	Karaj, Iran
UTPF 5	*P. flourescens*	Turnip	Karaj, Iran
UTPF 7	*P. flourescens*	Turnip	Karaj, Iran
UTPF17	*P. flourescens*	Wheat	Karaj, Iran
UTPF18	*P. flourescens*	Wheat	Karaj, Iran
UTPF24	*P. flourescens*	Wheat	Karaj, Iran
UTPF 27	*P. flourescens*	Wheat	Karaj, Iran
UTPF30	*P. flourescens*	Wheat	Karaj, Iran
UTPF32	*P. flourescens*	Wheat	Karaj, Iran
UTPF45	*P. flourescens*	Bean	Khomein, Iran
UTPF54	*P. flourescens*	Bean	Khomein, Iran
UTPF59	*P. flourescens*	Boll	GharehTapeh, Iran
UTPF60	*P. flourescens*	Rice	ShamukAlamut, Iran
UTPF61	*P. flourescens*	Rice	AlamuGhazvin, Iran
UTPF63	*P. flourescens*	Turnip	Mazandaran, Iran
UTPF65	*P. flourescens*	Turnip	Mazandaran, Iran
UTPF 68	*P. flourescens*	Turnip	Mazandaran, Iran
UTPF75	*P. flourescens*	Rice	Alamu, Iran
UTPF76	*P. flourescens*	Rice	Alamu, Iran
UTPF81	*P. flourescens*	Rice	Ghasinrood, Iran
UTPF83	*P. flourescens*	Rice	Alamu, Iran
UTPF85	*P. flourescens*	Rice	Gazerkhan, Iran
UTPF86	*P. flourescens*	Rice	Shamuk, Iran
UTPF87	*P. flourescens*	Rice	Razmian, Iran
UTPF88	*P. flourescens*	Rice	Alamu, Iran
UTPF90	*P. flourescens*	Rice	Shamuk, Iran
UTPF92	*P. flourescens*	Rice	Alamu, Iran
UTPF93	*P. flourescens*	Rice	Alamu, Iran
UTPF94	*P. flourescens*	-	India
UTPF95	*P. flourescens*	-	India

treatment can promote uniform germination and improve the plant growth associated with bacterial coatings (Bennett, 1998). There is no specific study neither for selecting effective biological agents on sunflower nor a useful way for applying them on seed. The objectives of this study were (1) screening and comparing the efficacy of the 30 strains of *Pseudomonas fluorescens* on enhancement of seed germination and seedling growth and selection of efficient strains (2) studying the effect of sunflower seed priming, biopriming and inoculation of seed with selected strains on the seed germination and seedling growth.

Materials and methods

Bacterial Screening

Thirty strains of *Pseudomonas fluorescens* were obtained from the Biological Control laboratory of Tehran University, Iran, and each of thirty strains named respectively started with UTPf (Table 1).

Bacterization of seed

One seed lot of sunflower cv. Azargol was used in the experiments. Seeds were surface-sterilized in 1.5% NaOCl for 5 minutes (Chen and Wu, 1999). For coating of seed with bacterial strains, each of bacterial strains was suspended in amounts of 1% solution of MC to achieve approximately 1×10^6 cfu.ml^{-1} suspensions. For each of bacterial strains, one hundred fifty surface-sterilized seeds were soaked in the relative bacterial suspension for three hours and also one hundred fifty surface-sterilized seeds were soaked in MC solution as control treatment. Then the seeds were surface dried under the sterile conditions. Effects of thirty bacterial treatments were compared with seeds treated just with MC in two individual experiments including laboratory germination test and greenhouse experiments.

Germination test

Treated seeds were placed on 9 cm petri dishes between papers (two papers under and one on the seeds) and wetted with 10 ml sterilized distilled water. Petri dishes were placed in an incubator in dark at 20±1°C for ten days. Thirty bacterial treatments and one control were placed in three replications including fifty seeds in each replication, arranged as a completely randomized design. Germination was considered to have occurred when the seed developed at least 2 mm long radical. In order to evaluate the germination rate, the germinated seeds were counted and removed from the petri dishes every twelve hours. 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 index, percentage and rate as well as vigor index were calculated using the following equations (Alvarado et al., 1987; Ruan et al., 2002; Ellis and Roberts, 1981):

Germination index (GI) = Σ (Gt/Tt), where Gt is the number of seeds germinated on day t and Tt is the number of days from beginning of germination test.

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

Germination rate (GR) = ΣNi / ΣTi Ni, where Ni is the number of newly germinated seeds at time Ti.

Vigor index (VI) = SDW G%, where SDW is seedling dry weight at the end of test and G% is the final germination percentage. These four indices for thirty treatment and one

Table 2. Effects of 30 bacterial seed treatments on germination index, germination percentage, germination rate and vigor index after 10 days.

Bacterial strain	germination index	germination percentage	germination rate	vigor index
UTPf2	10.51 fghij	90 abc	0.298 efg	7.5 efghij
UTPf5	8.85 ijk	75.55 cde	0.312 cdefg	6.84 ij
UTPf7	10.44 fghijk	90 abc	0.263 gh	9.329 abcdefgh
UTPf17	13.02 def	93.33 a	0.309 cdefg	8.36 cdefghij
UTPf18	16.08 abc	93.33 ab	0.364 abcde	9.88 abcde
UTPf24	10.33 fghijk	86.67 abcd	0.299 defg	8.53 bcdefghi
UTPf27	10.25 ghijk	92.22 ab	0.300 defg	7.98 defghij
UTPf30	8.93 hijk	90 abc	0.249 gh	10.33 abcd
UTPf32	10.44 fghijk	90 abc	0.263 gh	9.65 abcdefg
UTPf45	12.24 defg	86.67 abcd	0.343 bcdef	8.57 bcdefghi
UTPf54	7.75 k	65.56 e	0.310 cdefg	5.97 j
UTPf59	8.28 jk	72.32 de	0.291 fg	6.95 hij
UTPf60	10.25 ghijk	92.22 ab	0.300 defg	7.98 defghij
UTPf61	10.97 fghij	85.56 abcd	0.305 cdefg	10.67 abc
UTPf63	13.02 def	93.33 a	0.309 cdefg	8.45 bcdefghi
UTPf65	8.73 ijk	94.44 a	0.215 h	9.16 abcdefghi
UTPf68	8.93 hijk	90 abc	0.249 gh	7.37 fghij
UTPf75	10.44 fghijk	90 abc	0.263 gh	9.76 abcdef
UTPf76	16.95 a	94.44 a	0.411 a	11.64 a
UTPf81	16.56 ab	93.33 ab	0.399 ab	10.93 ab
UTPf83	10.19 ghijk	88.88 abc	0.312 cdefg	10.69 abc
UTPf85	14.23 bcd	92.22 ab	0.308 cdefg	10.48 abcd
UTPf86	11.09 fghi	87.77 abc	0.301 defg	10.65 abc
UTPf87	11.79 defg	92.22 ab	0.332 cdef	9.71 abcdefg
UTPf88	11.58 efgh	90 abc	0.278 fgh	9.81 abcdef
UTPf90	12.24 defg	86.67 abcd	0.343 bcdef	10.47 abcd
UTPf92	12.71 defg	94.44 ab	0.295 fg	10.14 abcd
UTPf93	14.07 cde	90 abc	0.366 abcd	8.09 defghij
UTPf94	8.85 ijk	75.55 cde	0.312 cdefg	8.99 bcdefghi
UTPf95	8.73 ijk	94.44 a	0.215 h	9.212 abcdefghi
Control*	11.93 defg	81.67 bcde	0.368 abc	7.24 ghij

In each column, values followed by different letters differ significantly at $P = 0.01$ according to Duncan's multiple range test
*Seed treated just with methyl cellulose as control

control were analyzed using analyzes of variance (ANOVA). Duncan's multiple range tests was used to compare means.

According to the results, the bacterial strains which caused the highest amount of studied indices were selected.

Greenhouse experiment 1

Treated seeds with thirty bacterial strains were sown in field soil moistened with tap water (3:1, vol/vol). Plastic boxes (11 × 17 cm) were filled with 200 gr of moist soil, and 3 seed were planted in 1-cm-deep holes and covered with 50 gr of moist soil. Each treatment was replicated three times and arranged in a completely randomized design. Planted boxes were placed in a growth chamber at 25 ± 1°C, with a twelve hours photoperiod at 200 $\mu m.m^{-1}.s^{-1}$ light intensity and 65-70% mean humidity. Seedlings were watered each day with tap water. For screening bioassays, the seedlings were removed after twenty days and root length, shoot height, dry and wet weight of seedlings were measured and data were analyzed using analyzes of variance (ANOVA) and Duncan's multiple range test was used to compare means. According to the results, the bacterial strains which caused the highest amounts of studied indices were selected.

Greenhouse experiment 2

In this experiment the effects of twelve selected strains in the expt. 1 were further investigated on increasing some of the growth factors. Large size test tubes were filled with half-strength water agar. Two treated seeds, containing 1×10^6 $cfu.ml^{-1}$ suspensions of selected bacterial strains and untreated seeds as control were placed onto the medium under the sterile conditions. The planted tubes were sealed and placed in the incubator in dark at 25±1°C. After germination of planted seeds, one of them was removed and then tubes were placed in a condition of twelve hours photoperiod at 200 $\mu m.m^{-1}.s^{-1}$ light

Fig 1. Effects of 3 treatments on the growth factors of sunflower seedlings. Primed, bioprimed and bacterizated seeds with the selected strains (UTPF76&86) and non treated seeds as control were sown in the field soil and the seedlings were removed after 15 days.

Table 3. Effects of 30 bacterial treatments on shoot height, root length, dry weight and wet weight of sunflower seedlings 20 days after planting in greenhouse.

Bacterial strain	Shoot height(cm)	Root length(cm)	Wet weight(gr)	Dry weight(gr)
UTPf2	18 abc	30.3 ab	4.1 bcde	0.227 bcdefgh
UTPf5	14.3 cdegh	26.3 abcdef	3.9 bcdef	0.26 abcde
UTPf7	12. 7 degh	26.3 abcdef	3.5 cdefghij	0.197 cdefghi
UTPf17	14.3 cdegh	29.7 abc	4.7 abc	0.263 abcd
UTPf18	14.3 cdegh	26 bcdef	3.6 bcdefghi	0.207 bcdefghi
UTPf24	20 ab	27.7 abcd	3.6 bcdefghi	0.24 bcdefg
UTPf27	14. 7 cdegh	28.3 abcd	3.7 bcdefgh	0.22 bcdefghi
UTPf30	12. 7 degh	28.3 abcd	4.8 ab	0.253 bcdef
UTPf32	13.3 degh	25.3 bcdef	3.8 bcdefg	0.213 bcdefghi
UTPf45	17 abcd	29.3 abc	4.1 bcde	0.253 bcdef
UTPf54	11 h	25.7 bcdf	2.7 efghij	0.167 ghi
UTPf59	12 egh	27.3 abcd	2.6 fghij	0.17 ghi
UTPf60	14.3 cdegh	28.3 abcd	3.7 bcdefgh	0.187 cdefghi
UTPf61	17 abcd	28.3 abcd	3.2 defghij	0.2 bcdefghi
UTPf63	16 bcde	28.3 abcd	4.4 abcd	0.233 bcdefg
UTPf65	11.3 gh	23.7 defg	2.5 ghij	0.15 hi
UTPf68	11. 7 gh	27 abcdef	3.3 defghij	0.143 i
UTPf75	13.7 cdegh	26 bcdef	3.4 defghij	0.193 cdefghi
UTPf76	14 cdegh	29 abc	3.8 bcdefg	0.28 ab
UTPf81	12. 7 degh	27.7 abcd	3.2 defghij	0.173 fghi
UTPf83	13.3 degh	26 bcdef	3.4 defghij	0.173 fghi
UTPf85	16.3 bcde	26.7 abcdef	3.7 bcdefgh	0.213 bcdefghi
UTPf86	21 a	31.3 a	5.5 a	0.33 a
UTPf87	17 abcd	27 abcdef	2.8 efghij	0.177 efghi
UTPf88	13. 7 cdegh	20.3 g	2.2 j	0.197 cdefghi
UTPf90	21 a	28.7 abcd	4.2 bcd	0.267 abc
UTPf92	20 ab	25 def	2.3ij	0.14 i
UTPf93	15. 7 cdeg	22 fg	2.4 hij	0.183 defghi
UTPf94	14 cdegh	30 ab	4.3 abcd	0.247 bcdefg
UTPf95	11.7 gh	28.3 abcd	3.5 cdefghij	0.26 abcd
Control*	11.3 gh	20.3 fg	2.6 fghij	0.147 hi

In each column, values followed by different letters differ significantly at $P = 0.01$ according to Duncan's multiple range test
*Seed treated just with methyl cellulose as control

intensity and 65-70% humidity. After fifteen days, shoot length, lateral roots length and numbers of lateral roots were measured. This experiment was done in a completely randomized design with twelve treatments and one control in three replication. According to the results of ANOVA and Duncan's multiple range test, the bacterial strain which caused the highest amount of studied factors was selected.

Osmopriming and biopriming

Seeds of sunflower were primed in 0.1% NaCl solution (the ratio of seed weight to solution volume was 1:5 gr.mL^{-1}) for twelve hours (Hussain et al., 2006). Seed were then washed with sterilized distilled water after primming and surface-dried for about one hour. For biopriming with *P. fluorescens*, the inoculums of two selected strains (from the germination test and the greenhouse expt.2) of *P. fluorescens* were applied during seed primming (2.5 gr of inoculums per 500 ml of water). After biopriming, seeds were dried in a laminar hood overnight at 22 ± 3°C. Treated seeds including osmoprimed, bioprimed, bacterizated seeds with the two selected strains and non treated seeds were sown in field soil moistened with tap water (3:1, vol/vol). Plastic boxes (11 × 17 cm) were filled with 200 gr of moist soil, and three seed were planted in 1cm-deep holes and covered with 50 gr of moist soil. Each treatment was replicated three times and arranged in a completely randomized design. Planted boxes were placed in a growth chamber at 25 ± 1°C, with a twelve hours photoperiod at 200 µm.m^{-1}.s^{-1} light intensity and 65-70% mean humidity. Seedlings were watered each day with tap water. In order to determine the effect of biopriming on the growth factors of sunflower seedlings, the seedlings were removed after fifteen days and root length, shoot height and wet weight of seedlings were measured. Data were analyzed using analyzes of variance (ANOVA) and Duncan's multiple range test was used to compare means.

Results

Bacterial Screening

To select strains of *P. fluorescens* which were more affective in seed invigoration and seedling growth, we arranged three experiments including a laboratory germination test and two greenhouse experiments, and after comparing some traits in each test we reached these following results.

Germination Test

In this test, four characteristics of germinating seeds including germination index, percentage, and rate and vigor index in response to strains of *P. fluorescens* were studied. Among the 30 strains used in this experiment, UTPf81, UTPf76 and UTPf17 in germination index; UTPf65, UTPf95, UTPf76, UTPf63 and UTPf17 in germination percentage and UTPf76, UTPf81, UTPf83, UTPf61, UTPf86, UTPf30, UTPf90, UTPf92, UTPf18, UTPf88 and UTPf75 in vigor index were significantly (P=0.01) different from the control treatments.

Table 4. Effects of 12 selected strains on shoot height, length of lateral roots and numbers of lateral roots of seedlings

Bacterial strain	Shoot height(cm)	length of lateral roots(cm)	numbers of lateral roots
UTPf2	16.3 abc	0.7 ef	80 b
UTPf5	16.5 abc	0.8 ef	42.6 f
UTPf17	11.8 de	2.4 c	60.6 d
UTPf24	16.2 abc	2.9 b	74.7 cb
UTPf30	16.7 abc	0.7 ef	50 e
UTPf45	9.5 e	1.1 de	32 g
UTPf63	16 abc	0.5 f	30 b
UTPf76	19.7 a	3.3 b	80 b
UTPf86	20 a	4.2 a	89.3 a
UTPf90	19 ab	3.8 a	70 c
UTPf94	14.7 cd	2 c	50.6 e
UTPf95	18.8 ab	1.3 d	50.6 e
Control*	15.2 bcd	2 c	40 f

In each column, values followed by different letters differ significantly at $P = 0.01$ according to
Duncan's multiple range test
*Seed treated just with methyl cellulose as control

UTPf76 and UTPf81 had the highest impact and value on germination rate but not significantly different comparing with control. The calculation of germination index showed that UTPf76 resulted in the highest (16.94) and UTPf54 resulted in the lowest values (7.75) and control was 11.93. UTPf65, UTPf95, UTPf76 and UTPf63 were the bests (94.5%), UTPf54 was the worst (65.56%) and control was 81.67% in germination percentage. In germination rate, UTPf76 resulted in the highest amount (0.41) and the least amount resulted from UTPf95 (0.21), while control was 0.36. The UTPf76 showed the highest value (11.64), UTPf54 the lowest (5.96) in vigor index while control was 7.241.

According to these results, UTPf76 significantly ($P= 0.01$) increased all four studied indices comparing to untreated control and was common in the first level. Therefore, it was selected as the best strain for seed invigoration (Table 2).

Greenhouse expt. 1

Thirty bacterial strains used in this experiment for comparing some seedling traits such as root length, shoot height, dry and wet weight in greenhouse. Among them, UTPf90, UTPf86, UTPf24 and UTPf95 in shoot height; UTPf86, UTPf94, UTPf12, UTPf17, UTPf45 and UTPf76 in root length; UTPf86, UTPf76, UTPf90, UTPf17, UTPf95 and UTPf5 in dry weight of seedling and UTPf86, UTPf30, UTPf17, UTPf63 and UTPf94 in wet weight of seedling showed the higher values and significantly ($P= 0.01$) increased studied indices over the other strains and untreated control (Table 3). So, UTPf86, UTPf76, UTPf90, UTPf17, UTPf95, UTPf5, UTPf45, UTPf30, UTPf2, UTPf24, UTPf63 and UTPf94 were selected for the next experiment.

Greenhouse expt. 2

Twelve bacterial strains which were selected from greenhouse expt. 1 were screened in greenhouse test 2. Among these, strains UTPf86 significantly ($P= 0.01$) increased studied indices, such as shoot length (20 cm), lateral roots length (4.23 cm) and numbers of lateral roots (89) over the other strains and untreated control (respectively: 15.16 cm, 2 cm and 40) and also was at the highest value in all three indices. So the most consistently performing strain which was selected according to its effect on improving seedling growth was UTPf86 (Table 4). In these experiments, two strains (UTPf76 and UTPf86) out of thirty, significantly ($P= 0.01$) increased studied indices such as germination index, percentage, rate as well as vigor index of seed; root length, shoot height, dry and wet weight of seedling over that of the other strains and controls. So UTPf76 and UTPf86 were selected for next experiment.

Osmopriming and Biopriming

In this experiment the effects of biopriming, osmopriming and bacterization of sunflower seeds on the growth factors of seedlings such as root length, shoot height and wet weight of seedlings were determined (Fig. 1). Biopriming with selected strains (UTPf76 and UTPf86) significantly ($P= 0.01$) increased the studied growth indices including root length, shoot height and wet weight of seedlings over the osmopriming, bacterial treatment and the untreated control (Fig. 2). This treatment resulted in the highest amount of shoot (28.2 cm), root height (35.9 cm) and seedling weight (8.9 g).

Discussion

Various strains of *P. fluorescens* have been found to be effective in plant growth promotion (Raj et al., 2004; Weller and Cook, 1983; Elliott et al., 1984; Miller et al., 1990; Kloepper et al., 1991). These PGPR inhabit plant roots and affect plant growth promotion by mechanisms such as increased solubilization and uptake of nutrients and/or production of plant growth regulators (Kloepper et al., 1989; Arshad and Frankenberger, 1991). So in this research we directed our efforts to select strains of *P. fluorescens* which were more effective on seed invigoration and improvement of seedling growth. Such studies are considered an efficient way to screen rhizobacteria for selection of PGPR strains (Khalid et al., 2001). Out of thirty bacterial strains tested in three experiments, two significantly ($P= 0.01$) increased studied indices such as: vigor index, mean germination time, germination rate and germination index of seed; root length, shoot height, dry and wet weight of seedling over that of the other strains and controls. So the most consistently performing strain in seed invigoration and improving seedling growth were UTPf76 and UTPf86 which were selected for the next experiment. Several studies on seed germination and seed emergence revealed the beneficial effects of seed priming by several ways such as heat, smoke, soaking, leaching, temperature, scarification and NaCl salinity (Ahmed et al. 2006). Solid matrix priming improved germination of hot pepper seed by 10–16% depending on temperature, and this effect enhanced when SMP was followed by halopriming and osmopriming (Pandita et al., 2007). Moreover, hydropriming (48 h) for tomato and solid-matrix priming (80% water holding capacity, 3 days) for eggplant and chilli were established as best methods of priming treatment capable of improving seed vigor (Venkatasubramanian and Umarani, 2007). On the other hand, application methods can influence the density and uniformity of microorganisms on a seed. As our results shows, priming is a promising techniques for uniformly applying microorganisms to crop seeds (Harman and Taylor, 1988; Khan, 1992; Bennett et al., 1992; Kubik, 1995; Pill, 1995; Warren and Bennett, 1997). In several cases, inoculation of seeds with biological agents in combination with priming has been reported to enhance and stabilize the efficacy of biological agents (Callan et al., 1990, 1991; Harman et al., 1989; Warren and Bennett, 1999). As we showed in this research bio-osmopriming treatment could promote rapid and more uniform germination under a wider range of soil temperatures while providing disease resistance and improved growth associated with bacterial coatings (Bennett, 1998).

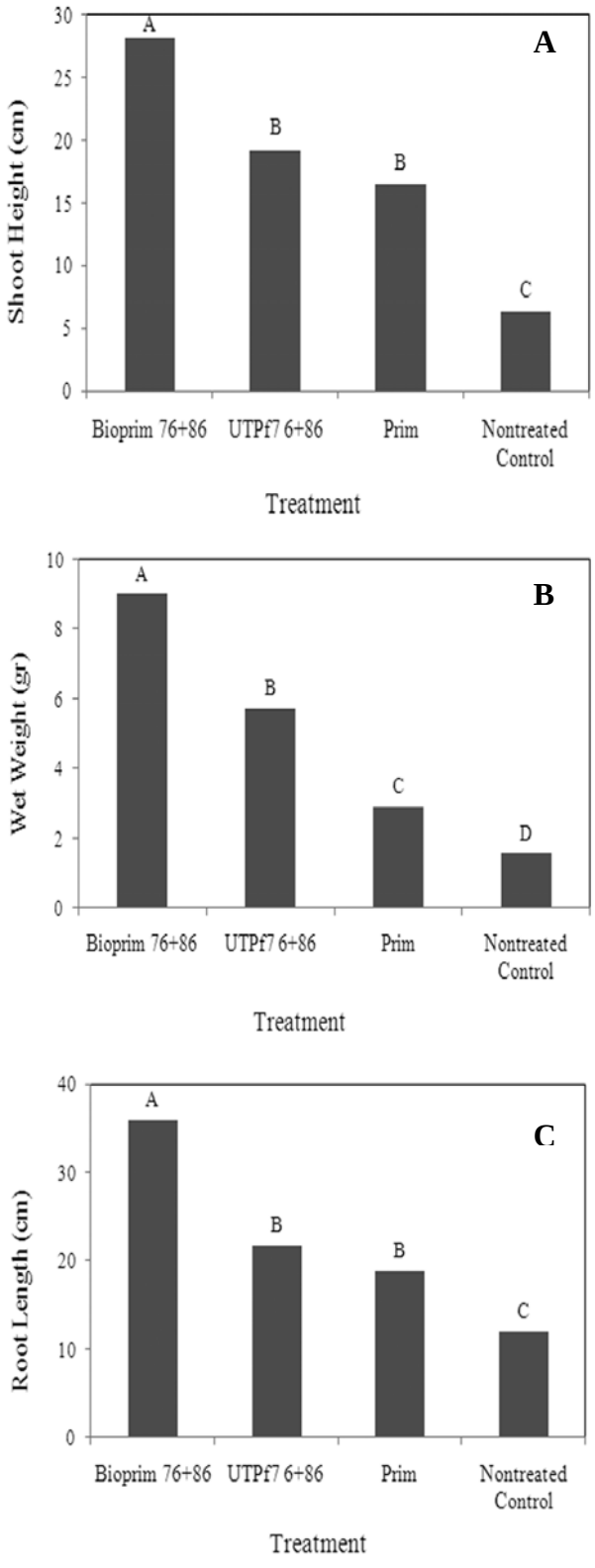

Fig 2. **A to C,** Effects of biopriming on the growth factors of sunflower seedlings. Osmoprimed, bioprimed and bacterizated seeds with the selected strains in the germination test and the greenhouse test and non treated seeds as control were sown in the field soil, the seedlings were removed after 15 days and shoot height (A), root length (B) and wet weight (C) of seedlings were measured. Treatments labeled with different letters differ significantly at $P = 0.01$.

Biopriming with selected strains (UTPf76 and UTPf86) significantly ($P = 0.01$) increased the studied growth indices including root length, shoot height and wet weight of seedlings over the osmopriming, bacterial treatment and the untreated control. It may be a result of good establishment and adherence of bacteria on the seed before planting, so they can properly colonize the seed and affect these traits. These experiments showed that inoculation of seeds with biological agents in combination with priming enhanced and stabilized the efficacy of biological agents and this result has previously been reported (Callan et al., 1990, 1991; Harman et al., 1989; Warren and Bennett, 1999). During biopriming, bacterial population on seed increased substantially, for example, 1×10^9 cfu seed^{-1} increased to 5×10^9 cfu seed^{-1} after biopriming (Wright et al., 2003). We used osmopriming as an application method to load the bacterial strains onto the seed. The value of biopriming over the seed bacterization alone was clearly observed. When osmopriming was combined with bacterial seed inoculation, root length, shoot height, dry weight and wet weight of seedlings in greenhouse was greatly increased comparing to osmopriming alone, means that application of *P. fluorescens* UTPf76&86 to dry seed was considerably less effective than biopriming with these organisms. Bioprimed seeds emerged at a more rapid rate than control. This indicates that relatively low bacterial levels may have impact to increase the bacterial population during the biopriming process which is an advantage. Seed hydration with no additional treatment improved emergence compared with untreated seed and but to a lesser extent with biopriming. The same result was reported in a research by Callan et al. (1990) that reported seed hydrated after treatment with MC emerged at a higher rate than seed hydrated after only surface-disinfestation.

Conclusions

The present study suggests that biopriming with *P. fluorescens* UTPf76 and 86 strains enhance the ability of sunflower seeds to invigorate and seedlings to grow uniformly. It can be concluded that microorganisms have the potential to proliferate, colonize and producing PGR's during priming procedures. At last, this technology may be of interest and value to growers who want to avoid chemical fertilizer of their seed.

Acknowledgments

We thank laboratory of Biological Control of Tehran University, Iran, for delivering bacterial strains. Also, we appreciate the donation of seeds by the institute of Seed and Plant Certification and Registration Research of Agricultural Research, Education and extension Organization.

References

Ahmed AK, Johnson KA, Burchett MD, Kenny BJ (2006) The effects of heat, smoke, leaching, scarification, temperature and NaCl salinity on the germination of *Solanum centrale* (the Australian bush tomato). Seed Sci Technol 34: 33-45

Alvarado AD, Bradford KJ, Hewitt JD (1987) Osmotic priming of tomato seeds, Effects on germination, field emergence, seedling growth and fruit yield. J Am Soc Hortic Sci 112: 427-432

Arshad M, Frankenberger WT (1991) Microbial production of plant hormones. In: Keister BD, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Netherlands

Ashraf M, Foolad MR (2005) Pre-sowing seed treatment – A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv Agron 88: 223-271

Basra SMA, Zia MN, Mehmood T, Afzal I, Khaliq A (2003) Comparision of different invigoration techniques in wheat *Triticum aestivum* L. seeds. Pak J Arid Agr 5: 11-17.

Bennett MA (1998) The use of biologicals to enhance vegetable seed quality. Seed Technology 20: 198-208

Bennett MA, Fritz VA, Callan NW (1992) Impact of seed treatments on crop stand establishment. Horttechnology 2: 345-349

Callan NW, Mathre DE, Miller JB (1990) Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in *sh2* sweet corn. Plant Dis 74: 368-372

Callan NW, Mathre DE, Miller JB (1991) Yield performance of sweet corn seed bioprimed and coated with *Pseudomonas fluorescens* AB254. HortScience 26: 1163-1165

Chen TW, Wu WS (1999) Biological control of carrot black rot. J Phytopathol 147: 99-104

Elliott LF, Lynch JM (1984) Pseudomonades as a factor in the growth of winter wheat *Triticum aestivum* L. Soil Biol Biochem 16: 69-71

Ellis RA, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. Seed Sci Technol 9: 373-409

Halmer P (2000) Commercial seed treatment technology. In: Black M, Bewley JD (eds) Seed Technology and its Biological Basis. Sheffield Academic Press, Sheffield

Halmer P (2003) Methods to improve seed performance. In: Benech-Arnold RL, Sanchez RA (eds) Seed Physiology, Applications to Agriculture. Food Product Press, New York

Harman GE, Taylor AG (1988) Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78: 520–525

Harman GE, Taylor AG, Stasz TE (1989) Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatment. Phytopathology 73: 631-637

Hussain M, Farooq M, Basra SMA, Ahmad A (2006) Influence of seed priming techniques on the seedling establishment, yield and quality of hybrid sunflower. Int J Agr Biol 8: 14-19.

International Seed Testing Association (1985) International Rules for Seed Testing. Seed Sci Technol, 13, Supplement, 307pp

Iswandi A, Bossier P, Vandenabeele J, Verstraete W (1987) Effect of seed inoculation with the rhizopseudomonads strain 7NSK2 on root micro biota of maize *Zea mays* and barley *Hordeum vulgare.* Biol Fert Soils 3: 153-158

Javed M, Arshad M, Hussain A (1996) Improving growth and yield of wheat with plant growth promoting rhizobacteria. Pak J Soil Sci 12: 95-100

Khalid A, Arshad M, Zahir ZA, Khaliq A (1997) potential of plant growth promoting rhizobacteria for enhancing wheat *Triticicum aestivum* L. yield. J Anim Plant Sci 7: 53-56

Khalid A, Arshad M, Zahir ZA (2001) Factors affecting auxin biosynthesize by wheat and rice rhizobacteria. Pak J Soil Sci 21: 11-18

Khan AA (1992) Preplant physiological seed conditioning. Hort Reviews 14: 131-181

Kloepper JW, Zabblotowitcz RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Netherlands

Kloepper JW, Lifshitz R, Zabblotowitcz RM (1989) Free living bacteria inocula for enhancing crop productivity. Trends in Biotechnology 7: 39-44

Korkmaz A (2006) Ameliorative effects of ethylene precursor and polyamines on the high temperature inhibition of seed germination in lettuce (*Lactuca sativa* L.) before and after seed storage. Seed Sci Technol 34: 465-474

Kubik KK (1995) Seed priming and survival of desiccated *Enterobacter cloacae* inoculated during seed priming. PhD Thesis, University of Nebraska, Lincoln

Lifshitz R, Kloepper JW, Kozlowksi M, Simon C, Carlson J, Tipping B, Zaleska I (1987) Growth promotion of canola rapeseed seedling by a strain of *pseudomonas putida* under gnotobiotic condition. Can J Microbiol 33: 390-395

Miller HI, Liberach E, Henken G, Veen JA (1990) Fluctuations in the fluorescent pseudomonad and actinomycetes populations of rhizosphere and rhizoplane during the growth of spring wheat. Can J Microbiol 36: 254-258

Nascimento WM, Pereira RS (2007) Preventing thermo-inhibition in carrot by seed priming. Seed Sci Technol 35: 504-507

Pandita VK, Anand A, Nagarajan S (2007) Enhancement of seed germination in hot pepper following presowing treatments**.** Seed Sci Technol 35: 282-290

Pill WG (1995) Low water potential and presowing germination treatments to improve seed quality. In: Basra AS (ed) Seed quality, basic mechanisms and agricultural implications. Haworth Press Inc, New York

Ruan S, Xue Q, Tylkowska K (2002) The influence of priming on germination of rice *Oryzo sativa* L. seeds and seedling emergence and performance in flooded soil. Seed Sci Technol 30: 61-67

Raj N, Shetty N, Shetty H (2004) Seed biopriming with *Pseudomonas fluorescens* strains enhances growth of pearl millet plants and induces resistance against downy mildew. Int J Pest Manage 50(1): 41-48

Taylor AG, Harman GE (1990) Concepts and technologies of selected seed treatments. Annu Rev Phytopathol 28: 321–329

Venkatasubramanian A, Umarani R (2007) Evaluation of seed priming methods to improve seed performance of tomato (*Lycoperison esculentum*), egg plant (*Solanum melongena*) and chilli (*Capsicum annum*). Seed Sci Technol 35: 487-493

Warren JE, Bennett MA (1997) Seed hydration using the drum priming system. HortScience 32: 1220-1221

Warren JE, Bennett MA (1999) Bio-osmopriming tomato *Lycopersicon esculentum* Mill. seeds for improved stand establishment. Seed Sci Technol 27: 489-499

Weller DM, Cook RJ (1983) Suppression of take-all of wheat by seed treatment with fluorescent pseudomonads. Phytopathology 73: 463-469

Wright B, Rowse H, Whipps JM (2003) Microbial population dynamics on seeds during drum and steeping priming. Plant Soil 255: 631-640

Zahir ZA, Arshad M, Azam M, Hussain A (1997) Effect of auxin precursor L-tryptophan and *Azotobacter* inoculation on yield and chemical composition of potato under fertilized condition. J Plant Nutr 20: 754-752

Zahir ZA, Akram M, Arshad M, Khalid A (1998a) Improving maize yield by inoculation with plant growth promoting rhizobacteria. Pak J Soil Sci 15: 7-11

Zahir ZA, Arshad M, Khalid A (1998b) Promoting crop yield by Azotobacter inoculation. J Agr Res 36: 369-380