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Full Length Research Paper

Effect of seed priming with plant growth promoting Rhizobacteria (PGPR) on dry matter accumulation and yield of maize *(Zea mays* L.) hybrids

Raouf Seyed Sharifi[1], Kazem Khavazi[2] , Abdolghayoum Gholipouri[1]

1- College of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran
2- Research Institute of Soil and Water , Tehran ,Iran

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In order to evaluate the effects of seed priming with Plant Growth Promoting Rhizobacteria (PGPR) on dry matter accumulation and yield of maize *(Zea mays* L.) hybrids, A factorial experiment based on randomized complete block design with three replications was conducted in 2009 at the Research Farm of the Faculty of Agriculture University of Mohaghegh Ardabili. Factors were: seed priming with Plant Growth Promoting Rhizobacteria in three levels containing, without priming (as control), priming with *Azotobacter*, *Azosprilium* and *Azotobacter+Azosprilium* plus maize hybrids (SC-404, SC-410 and SC-434). The results showed that seed priming with Plant Growth Promoting Rhizobacteria affected grain yield, plant height, number of kernel per ear, number of grains per ear row significantly. Maximum of these characteristics were obtained by the plots which seeds were inoculated with *Azotobacter* bacteria. Mean comparison of treatment compound corn hybrids ×various levels of priming with PGPR showed that maximum grain yield and number of kernel per ear were obtained by the plots which was applied SC-434 hybrid with *Azotobacter* bacteria and minimum of it was obtained in SC-404 hybrid without of seed priming. Investigation of process of variances of dry matter accumulation indicated that in all of hybrids, it increased slowly until 36 days after sowing and then increased rapidly till 116 days after sowing and from 116 days after sowing till harvest time, it decreased due to increasing aging of leaves. In additional, in all of maize hybrids, dry matter accumulation in unit of area increased with seed priming with *Azotobacter*. The highest grain yield (7.01 ton/ha) and dry matter accumulation (2019 gr /m^2) was obtained in treatment compound SC-434 maize hybrid at seed priming with *Azotobacter*. Thus, it can be suggested that SC-434 hybrid should be inoculated with *Azotobacter* bacteria in conditions of Ardabil Plain.

Key words: Corn, Dry matter accumulation, PGPR and Yield.

INTRODUCTION

Every increasing population of the world demands the increase in food production which intern depends upon the improved agriculture practices. Corn (*Zea mays* L.) is one of the most important cereal crop grown principally during the summer season in Iran. Maize grain is used for both human consumption and poultry feed. This crop has much higher grain protein content than our staple food rice. Based on area and production, maize is the 3th most important cereal crop after wheat and rice in world. The yield of maize in Iran is very low as compared to other maize producing countries. One of the most important effective factors in increasing of corn yield is seed priming with plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and promote growth when added to seeds, roots or tubers have been termed plant-growth-promoting rhizobacteria (Kloepper et al., 1980) and increase plant growth and yield (Wu et al., 2005). Among them are

*Correspounding author Email: Raouf_ssharifi@Yahoo.com, Tel: 0098-914-3556585, Fax: 0098-0451-5512204

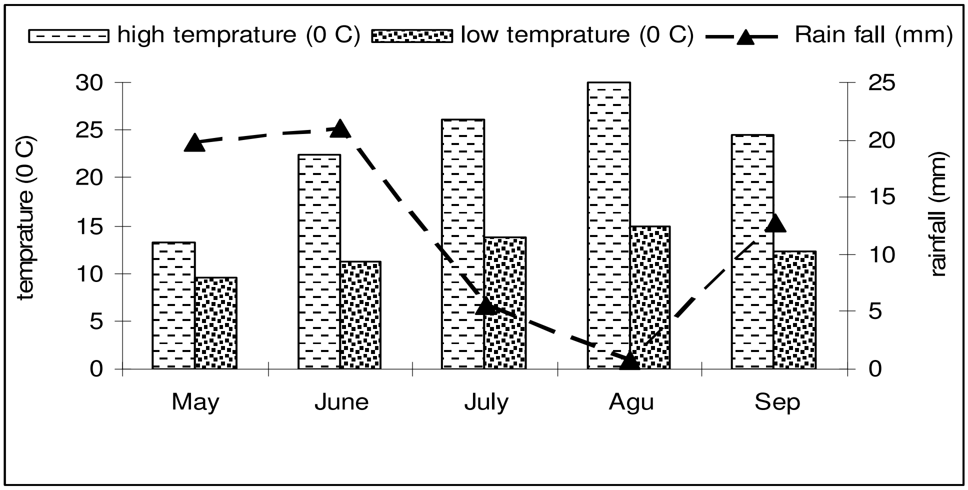

Figure 1. high and low temperatures and rainfall recorded during the period of corn growth (May –October) in 2009.

strains from genera such as *Azosprilium* and *Azotobacter* (Rodriguez and Fraga., 1999). The mechanisms by which PGPRs promote plant growth are not fully understood (Vessy, 2003). But, several mechanisms have been suggested by which PGPR can promote plant growth, including phytohormone production, enhancing stress resistance, N2 fixation, stimulation of nutrient uptake and biocontrol of pathogenic microorganisms (Rodriguez and Fraga., 1999; Sindhu et al.,1999), increasing the supply or availability of primary nutrients to the host plant (Wu et al., 2005), the synthesis of antibiotics, enzymes and fungicidal compounds (Ahmad et al., 2006; Bharathi et al., 2004 ; Jeun et al., 2004). Kloepper and Beauchamp (1994) have been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Bashan et al. (2004) and Cakmake et al. (2006) reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in total plant biomass, nutrient uptake, plant height, leaf size, leaf area index and root length of cereals (Bashan et al., 2004). Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported by Asghar et al. (2002) and Biswas et al. (2000). Kloepper et al (1980 a, b) reported that plant yields 10 to 30% increased in non-legume crops such as potato, radish, corn and sugar beet. Numerous studies have shown a substantial increase in dry matter accumulation and seed yield following inoculation with PGPR (Perveen et al., 2002 ; Wani et al., 2007). Dilfuza (2007) suggested that inoculation of corn seeds with *Azospirillum brazilance* increased dry matter accumulation. Omar (1998) reported a significant increase in the dry matter yield of wheat due to seed priming by PGPR. Zaidi and Khan (2005) have suggested that seed priming with PGPR increased dry matter accumulation and grain yield of wheat. *Azospirillum* inoculation increased dry matter by 40% in *Zea mays* and in *Triticum aestivum* (Bashan., 1998). Murty and Ladha (1988) found that inoculation of *A. lipoferum* to rice roots significantly increased shoot fresh and dry weights. Dobbelaere et al (2003) ; Cakmakı (2005a) have been reported that PGPR can increase yield and leaf area index, shoot and roor weight and delay leaf senescence. Trails with Plant growth-promoting rhizobacteria indicated that yield and dry matter accumulation increase in rice (Sudha et al, 1999), barley (Cakmakı et al., 2001 ; Fiahin et al., 2004), wheat (De Freitas., 2000; Cakmakı et al., 2007), maize (Pal, 1998), sugarcane (Sundara et al., 2002). There is little information available on effects of these organisms on corn under field conditions in Iran. Hence, the objectives of this study were to determine the effect seed inoculation (priming) with Plant Growth Promoting Rhizobacteria on dry matter accumulation and grain yield of maize *(Zea mays* L.) hybrids in condition of Ardabil Plain in Iran.

MATERIAL AND METHOD

A factorial experiment based on randomized complete block design with three replications was conducted in 2009 at the Research Farm of the Faculty of Agriculture University of Mohaghegh Ardabili (lat 38̊ 15′ N; long 48̊ 15′ E; Alt 1350m). Climatically, the area placed in the semi-arid temperate zone with cold winter and hot summer. Average rainfall is about 367 mm that most rainfall concentrated between winter and spring. Temperature mean and rainfall during the period of corn growth season (May-October), is presented in Figure 1. The soil was salty loam with EC about 3.61 ds/m, pH about 8.1 and SP about 43%. Before sowing of the crop the field was well prepared by plowing twice with tractor followed planking to make a fine seed bed. Treatments were seed priming with Plant Growth Promoting Rhizobacteria (PGPR) in three levels containing, without inoculation (as control), priming with

Table 1. Analysis of variance for the effects of seed priming with PGPR on studied traits in maize hybrids

		MS				
S.O.V	DF	Plant height	Number of kernels per ear	The number of grains rows	The number of grains per ear row	Grain yield
Replication	2	3152.27**	18729.85**	5.177**	25.64**	2.15**
Seed priming with PGPR	3	433.31**	2401.706**	.00297	.019	.711**
Maize hybrids	2	489.21**	2491.72**	3.22**	58.11**	3.08**
Seed priming with PGPR × Maize hybrids	6	.156	16.71*	.0056	.0185	.014**
Error	22	.48	4.06	.00409	.011.	.00107

*, ** and show significant differences at 0.05, 0.01 probability level, respectively.

Azotobacter, *Azosprilium* and *Azotobacter + Azosprilium* plus maize hybrids (SC-404, SC-410 and SC-434). For inoculation seeds were coated with gum Arabic as an adhesive and rolled into the suspension of bacteria until uniformly coated. The strains and cell densities of microorganisms used as PGPR in this experiment were 10^7 bacteri /gram. Row spacing was 75cm and distances between plants in the rows were 15.68 cm for the appropriate final stand of 85000 plants ha^{-1}. Seeds were placed at 5-6 cm depth. Plot size was 6 m × 3.75m with five rows per plot. Plots and blocks were separated by 1.5 m unplanted distances. Corn seeds were planted in the thirth week of May. Two seeds were sown per hill and at the third leaf stage , plants were thinned to one plant per hill. The bacterial strains *Azotobacter* and *Azosprilium* were isolated from the rhizospheres of corn. Based on soil test, fertilizer basic dose of N.P.K at the rate of 150-75-75 kg ha^{-1} were applied in the form of urea, triple super phosphate and nitrate potassium. All of phosphor and potassium were applied at the autumn. Nitrogen fertilize was applied as 1/3 at sowing, 1/3 at 4-6 leafy stage and 1/3 at reproductive stage (tasseling appearance). The field was immediately irrigated after planting. Weeds were controlled manually. All other agronomic operations except those under study were kept normal and uniform for all treatments. For estimation of dry matter accumulation, from .2 m^2 in each plot was sampled randomly in each treatment compound and average for recording the change in dry weight in shoots (above ground). Sampling intervals were ten days at different stages of the corn growth (36, 46, 56, 66, 76, 86, 96, 106, 116 and 126 days after sowing). For dry weight determination, samples were oven dried at 70 ±5° C to constant weight. The parameter of total dry matter (TDM) was determined with using of equation 1 according of method of Acuqaah (2002) and Gupta and Gupta (2005).

$$TDM = e^{a+bt+ct^2+dt^3} \quad (1)$$

In this equation, t is the intervals of sampling and a, b and c are coefficient of equation. Harvest sample was taken of 2 m long from the three middle rows for measuring grain yield. The other traits studied in this research were determined in the following ways:

Plant height: mature plant heights of 8 random plants/plot were measured in cm as the distances from ground level to the lowest branch of the panicle. Number of kernel per ear: the number of kernels in 8 ears was counted after they had been shelled, and was divided by the number of ears. The other characteristics such as number of grains per ear row and the number of grains rows were determined in the center three rows of each plot according to Ulger (1998). Analysis of variance and regression were performed using SAS computer software packages. The main effects and interactions were tested using the LSD test.

RESULTS AND DISCUSSION

Seed priming with PGPR: seed priming with PGPR had significant effects on grain yield, plant height, number of kernels per ear and number of grains per ear row. Maize hybrids had different response to these characteristics (Table 1).

Number of kernels per ear:

Data regarding the effect of maize hybrids and seed inoculation with PGPR on number of kernels per ear are given in table 2. The response of number of kernels per ear to seed inoculation with PGPR was significant (Table 1). The number of kernels per ear ranged between 361.99 and 390.8 in corn hybrids. Among of seed inoculation with PGPR, maximum number of kernels per ear was recorded to inoculation with *Azosprilium* (397.6) and minimum it was recorded at control treatment (357.9). Our results concur partly with observations made by Golami et al (2009), who reported that the kernels number increased with seed priming with PGPR. Increase in grains cob^{-1} with inoculation might be due to the positive response of corn at inoculation with PGPR. These results are also in agreement with De Freitas (2000) who concluded that grain number per ear in wheat was highest at inoculation with PGPR. Means comparison indicated that the maximum (390.8) number of kernels per ear was recorded for SC-434 hybrids and minimum value was recorded for SC-404 hybrids (361.99). Number of kernels per ear plays an important role to determining grain yield. Mean comparison of

Table 2. Means comparison for yield and some of agronomic characteristics corn hybrids maize as affected by seed priming with PGPR

characteristics / Treatments	Plant height (cm)	Number of kernels per ear	The number of grains rows	The number of grains per ear row	grain yield (ton/ha)
Seed priming with PGPR					
Without inoculation (as control)	189 c	357.9 c	-	28.17 c	5.72 c
Azotobacte	203.13 a	397.6 a	-	30.62 a	6.85 a
Azosprilium	197.92 b	373 b	-	29.7 b	6.17 b
Azotobacter + Azosprilium	192.55 b	377 b		29 b	5.87 c
Maize hybrids					
SC-404	187.93 c	361.99 c	15.07 c	27.23 c	5.37
SC-410	194.06 b	377.42 b	15.85 b	29.33 b	6.42 b
SC-434	200.7 a	390.8 a	16.07 a	31.63 a	6.83 a

Values followed by the different letters are significantly different

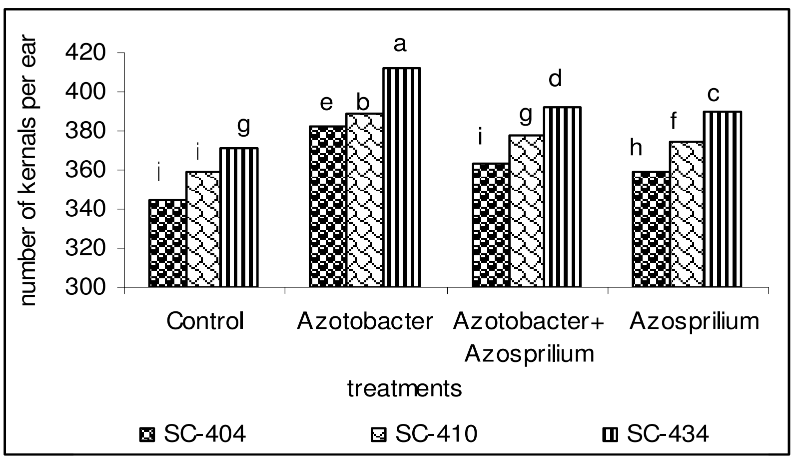

Figure 2. Effect of seed priming with PGPR on number of kernels per ear in maize hybrids

treatment compound corn hybrids ×various levels of priming with PGPR showed that maximum number of kernels per ear was obtained by the plots which was applied SC-434 with *Azotobacter* bacteria and minimum of it was obtained in SC-404 hybrid without of seed priming (figure 2).

Plant height

seed inoculation with PGPR significantly increased the plant height in maize hybrids. Data regarding the effect of maize hybrids and seed inoculation with PGPR on plant height are given in table 2. In general, the maximum plant height (203.12 cm) was obtained to seed inoculation with *Azosprilium*, while the least value (192.55) was recorded at without inoculation. Similar results have been reported by Bashan and Hulguin (2004) and Cakmake et al., (1996). They reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as plant height. Means of comparisons for maize hybrids indicated the maximum (200.7 cm) plant height was recorded for SC-434 hybrids and minimum value (187.93 cm) was recorded for SC-404 hybrid (table 2).

Table 3. Regression equations between dry matter accumulation of maize (Zea mays L.) hybrids and various levels of seed priming with Plant Growth Promoting Rhizobacteria (PGPR)

Treatment compound (maize hybrids × seed priming with PGPR)	Regression equations	
SC-404 × seed priming with Azotobacter	$TDM = e^{(-.552 + .195x - .00164x2 + .0000044x3)}$	.9
SC-404 × seed priming with Azotobacter + Azosprilium	$TDM = e^{(-.506 + .195x - .00164x2 + .0000044x3)}$	.84
SC-404 × seed priming with Azosprilium	$TDM = e^{(-.571 + .196x - .00164x2 + .00000448x3)}$	.87
SC-404 × without priming	$TDM = e^{(-.605 + .193x - .00159x2 + .0000044x3)}$	.92
SC-410 × seed priming with Azotobacter	$TDM = e^{(3.63 + .079x - .00061x2 + .0000016x3)}$	.89
SC-410 × seed priming with Azotobacter + Azosprilium	$TDM = e^{(3.82+ .077x - .00058x2 + .00000152x3)}$	.91
SC-410 × seed priming with Azosprilium	$TDM = e^{(3.86+ .0783x - .00059x2 + .00000149x3)}$	.9
SC-410 × without priming	$TDM = e^{(3.93 + .076x - .00058x2 + .00000153x3)}$	.87
SC-434× seed priming with Azotobacter	$TDM = e^{(-.154 + .18x - .0015x2 + .0000043x3)}$	.92
SC-434 × seed priming with Azotobacter + Azosprilium	$TDM = e^{(-.44 + .196x - .00167x2 + .00000482x3)}$	.88
SC-434 × seed priming with Azosprilium	$TDM = e^{(-.567 + .205x - .00173x2 + .00000491x3)}$	.84
SC-434 × without priming	$TDM = e^{(-67 + .208x - .00175x2 + .00000492x3)}$	.87

The number of grains rows

Data recorded on average the number of grains rows of maize hybrids is represented in table 2. Means comparisons indicated that maximum number of grains rows (16.07) was observed for SC-434 hybrids and minimum value (15.06) was observed for SC-404 hybrids. Similar results have been reported by Tetio–Kargho and Gardner (1988) who reported that the number of grains rows had significantly affected by maize hybrids.

The number of grains per ear row

Data regarding the effect of maize hybrids and seed priming with PGPR on the number of grains per ear row are given in table 2. The response of the number of grains per ear row to seed priming with PGPR was significant. Maximum (30.62) the number of grains per ear row was recorded seed priming with *Azotobacter* and minimum it was recorded at control treatment (28.17). Similar results have been reported by De Freitas (2000) and Cakmakı et al., (2007) in wheat and Pal (1998) in maize. Means comparisons indicated that maximum the number of grains per ear row (31.63) was observed for SC-434 hybrids and minimum value (27.23) was observed for SC-404 hybrids. Similar results have been reported by Tetio–Kargho and Gardner (1988) who reported that the number of grains per ear row of corn were significantly affected by maize hybrids.

Grain yield

Grain yield is the main target of crop production. The grain yield was significantly affected by both maize hybrids and seed priming with PGPR. Seed priming with PGPR significantly increased the grain yield. The grain yield varied between 5.72 ton/ha in without priming till 6.85 ton/ha in seed priming with *Azotobacter* (table 2). A similar trend in yield differences across seed priming with PGPR have been reported by Dobbelaere et al (2003) and Cakmakı (2005 a, b). They have been reported that PGPR can increase yield. Kloepper and Beauchamp (1992) have been shown that wheat yield increased up to 30% in seed priming with PGPR. Maximum grain yield was produced by SC-434 hybrid (6.83 ton /ha) while minimum by SC-404 hybrid (5.37 ton/he). Mean comparison of treatment compound corn hybrids ×various levels of priming with PGPR showed that maximum grain yield were obtained by the plots which was applied SC-434 with *Azotobacter* bacteria and minimum of it was obtained in SC-404 hybrid without of seed priming. Similar results have been reported by Cakmakı et al, (2001) in barley, De Freitas (2000) in wheat and Pal (1998) in maize . They reported that seed priming with PGPR increased grain yield.

Dry matter accumulation

Process of variances dry matter accumulation of treatment compounds corn hybrids× various levels of seed priming with PGPR have been showed in figures 4, 5 and 6. In addition regression equations have been given in table 3. Study of process of variances dry matter of corn hybrids× various levels of seed priming with PGPR (Figures 4, 5 and 6) showed that in all of hybrids, dry matter accumulation increased during plant growth in seed priming with PGPR and reached to a maximum level at 106-116 DAS, then showed a declining trend at maturity (116-126 DAS). Bashan et al. (2004) and Cakmake et al. (2006) reported that inoculation of plants with *Azospirillum* could result in significant changes in

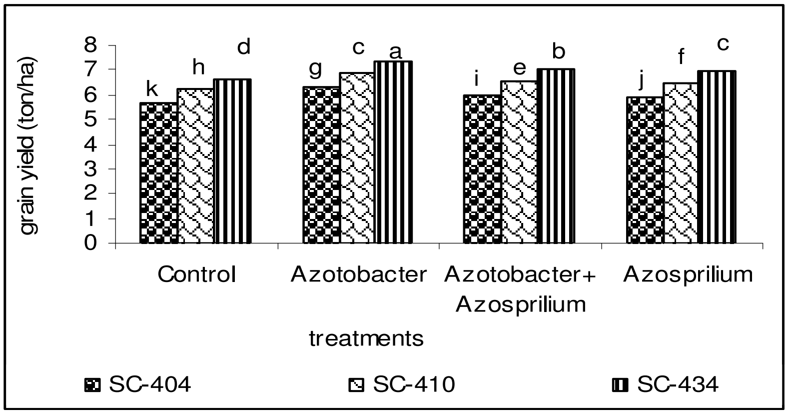

Figure 3. Effect of seed priming with PGPR on number of grain yield in maize hybrids

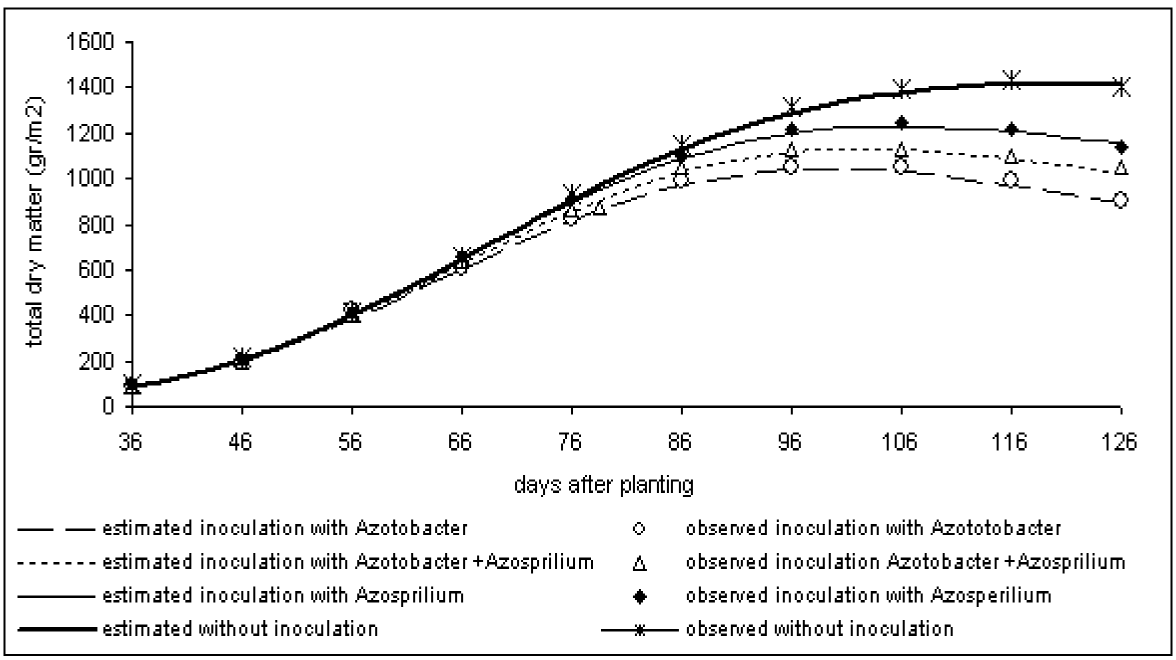

Figure 4. trend of variances dry matter accumulation of SC-404 hybrid in various levels of seed priming with PGPR bacteria

various growth parameters, such as increase in total plant biomass. Zaidi and Khan (2005) have suggested that seed priming with PGPR increased dry matter accumulation. The increase in dry matter accumulation with seed priming with PGPR indicates the favorable response of corn hybrids to seed priming with PGPR. Similar observations were also made by Golami er al. (2009) in corn. Perveen et al. (2002) ; Wani et al. (2007) have been reported increase in dry matter accumulation due to inoculation with PGPR. Study of dry matter accumulation trends of SC-434 hybrids in various levels of seed inoculation with PGPR shows that dry matter accumulation increased slowly until 36 days after sowing in seed priming with *Azotobacter* and then increased rapidly till 106-116 days after sowing. From 116 days after sowing till harvest time, accumulated dry matter decreased due to increasing aging of leaves and decreasing of net assimilation rate (Figure 4). On the other hand, dry matter accumulation in unit of area

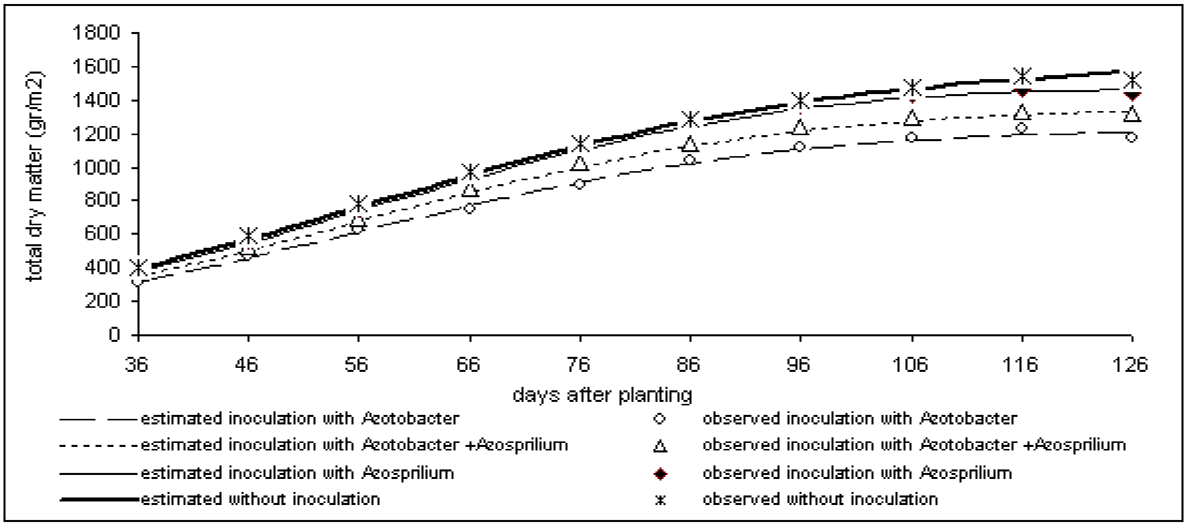

Figure 5. trend of variances dry matter accumulation of SC-410 hybrid in various levels of seed priming with PGPR bacteria

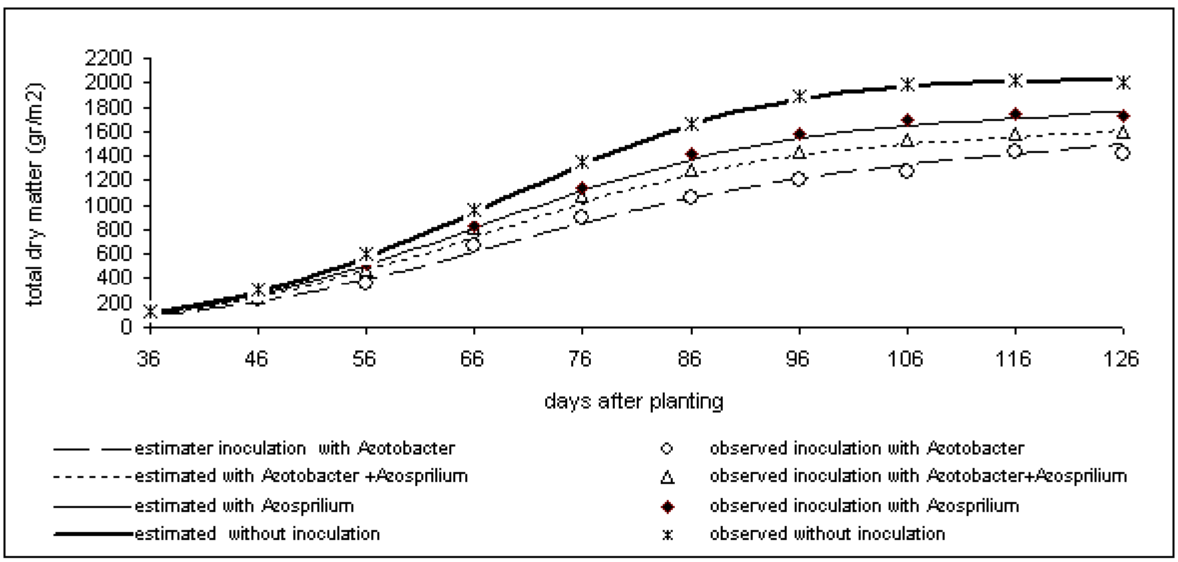

Figure 6. trend of variances dry matter accumulation of SC-434 hybrid in various levels of seed priming with PGPR bacteria

increased with seed inoculation with *Azotobacter*, as the maximum (2019 gr/m^2) and the minimum (1460.22 gr/m^2) biomass in unit of area obtained from seed inoculation with *Azotobacter* and without priming, respectively (Figure 4). Study the accumulated dry matter in other hybrids (SC-404 and SC-410) indicated that dry matter accumulation in these hybrids increased in seed inoculation with *Azotobacter* (Figure 5 and 6). Rokhzadi et al. (2008) reported that seed priming with plant growth-promoting rhizobacteria increased dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field conditions. Similar results have been reported by Pal (1998) in maize.

CONCLUSION

In this experiment, seed inoculation with PGPR showed significant effects on corn hybrids yield, yield components and dry matter accumulation. The highest grain yield and dry matter accumulation recorded at seed inoculation with Azotobacter with SC-434 hybrids. In conclusion, it can be suggested that seed inoculation with Azotobacter should be applied in SC-434 hybrids in conditions of Ardabil Plain in Iran.

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