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Agromorphological Responses of Forage Sorghum (*Sorghum bicolor* L.) to Different Growth Promoting *Pseudomonas fluorescence* Strains

[1]*Arash Shaterabadi*, [2]*Mohammad Reza Ardakani*, [3]*Kazem Khavazi*, [1]*Mahdi Changizi and* [4]*Saeed Mafakheri*

[1]Department of Agronomy and Plant Breeding, Arak Branch, Islamic Azad University, Arak, Iran
[2]Agriculture Research Center, Karaj Branch, Islamic Azad University, Karaj, Iran
[3]Soil and Water Research Institute, Karaj, Iran
[4]Young Researchers Club, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract: This study was conducted in 2009 at the Research Field of Islamic Azad University, Arak branch, Iran, in order to study the effect of different Pseudomonas strains and application methods on growth and yield of sorghum. The experimental design was factorial in the form of a randomized complete block design with three replications. Two factors of the experiment were three strains of *Pseudomonas* (*P. fluorescens* strains 87, 162 and 169 and the control without inoculation) and three application methods (seed inoculation, spray, seed + spray). Results indicated that strain, application method and their interaction significantly affected the measure traits. The highest stem length (164.33 cm) and fresh forage yield (48.10 ton/ha) were obtained in the interaction of strain 87 × the doubled inoculation (seed + spray).

Key words: Growth promotion · Bacterial inoculation · Forage · Sorghum

INTRODUCTION

Plant Growth Promoting Rhizobacterias (PGPR) is any rhizospheric bacteria which improve plant growth. *Pseudomonas* species are among the most important PGPR which improve plant growth directly and indirectly. These bacteria improve plant growth indirectly through the biological control and inhibition of plant pathogens [1,2]. Weller and Cook [3] found that *Pseudomonas fluorescens* strain Q72-a80 controlled wheat Pythium root rot. Klopper and Schroth [47] also represented that *P. fluorescens* strain B10 effectively inhibits barley wilt.

In addition to the indirect mechanisms, *Pseudomonas* species directly affect plant growth, as they can biologically fix nitrogen, increase the available phosphorus to plants, produce plant growth promoting hormones, improve plant root system development and enhance the activity of other beneficial soil microorganisms [5-7]. Gen and Jordan [8] reported that inoculating spring sown tomato with *Pseudomonas fluorescens* increased the weight of top quality fruits from 5.6% to 9.6% of the whole produced fruits. In fall sown tomato, the treatments increased the weight of top quality fruits by 18.2%. Moreover, fruit size was increased by 11.1% and unmarketable fruits were reduced to 12% (in treatment) from 23% (in the control). Suslow and Schroth [9] conducted experiments to evaluate the effect of *Pseudomonas fluorescence* on sugar beet under greenhouse and field conditions. In greenhouse, the bacterial inoculation increased root and shoot dry weight by 20-25% and in field experiments, root yield was increased by 6.1- 8.6% compared with the control. Klopper and Schroth [4] observed 144% improvement of radish yield as the result of PGPR inoculation. Ardakani *et al.* [10] also reported that inoculating wheat seeds with *Azospirillum brasilense*, *Streptomyces sp.* and *Mycorrhizae* (*Glomus intraradices*) significantly improved macronutrients absorption.

Some researchers represent that PGPR inoculation is more effective on plants with short growth period [11]. *Pseudomonas* inoculation improves plant nutrient absorption. Afzal and Asghari [12] reported an increased P absorption in wheat inoculated with *Pseudomonas*. The improved Pabsorption can be attributed to the

Corresponding Author: Mohammad Reza Ardakani, Agriculture Research Center, Karaj Branch, Islamic Azad University, Karaj, Iran.

production of plant growth regulators (PGR) by the bacteria, or reduction of soil pH because of the bacteria activity [13].

Pseudomonas has synergistic effect on the activity of other soil microorganisms. Tilak *et al.* [14] represented that the co-inoculation of *Pseudomonas putida* and *Rhizobium* strains affected growth, nodulation and enzymes activity in pigeon pea, better that the individual application. Finally, this experiment was conducted to find the best *Pseudomonas* strain and application method for improvement of sorghum growth and yield production.

MATERIALS AND METHODS

This study was conducted in 2009 at the Research Field of Islamic Azad University, Arak branch, Iran. The soil type was Clay loam; other soil properties are listed in Table 1.

The study was conducted in a factorial experiment in the form of a randomized complete block design (RCBD) with three replications and two factors:

Pseudomonas Strains: *P. fluorescence* strains 87, 162, 169 and the control without inoculation.

Pseudomonas Application Method: Seed inoculation, spray and seed + spray (double inoculation). For seed inoculation, 1 kg seed was mixed with 5 ml of CMC gum and then with 30 g of the inoculant powder. In spray method, 400 L/ha water solution containing 4 Li of the bacterium was sprayed on the required plots at 7-9 leaves stage.

When the field was being prepared 150 kg/ ha urea was applied in soil and on June 8th, sorghum (cv. Speed Feed) was planted. The speed feed cultivar is a fast growing one, with fast regrowth after harvests. At the end of the growing season, when the field was in 10% flowering, traits such as stem length, stem dry weight, the number of leaves, leaf dry weight, fresh and dry forage weigh were measured. To measure the stem, leaf and forage dry weight, the harvested plants were divided into two parts (stem and leaves), dried at 70°C oven for 24 h and weighted. Finally, data were analyzed using MSTAT-C and mean comparison was conducted according to the Duncan's multiple range test.

Table 1: Soil properties of the experimental field.

K (ppm)	P (ppm)	Total N (%)	O.C (%)	pH	EC (dS/m)
220	16.8	0.09	0.87	7.7	1.7

RESULTS AND DISCUSSION

Stem Length: Analysis of variances indicated that stem length was significantly affected by the strain ($p \leq 0.05$), application method and their interaction ($p \leq 0.01$; Table 2). Mean comparison indicated that the effect of three strains was not significant on this trait. The three application methods had also a non-significant effect on stem length (Table 3). However, the interaction between strain × application method significantly affected stem length and the highest value (164.33 cm) was occurred in strain 87 × double inoculation (seed + spray). The lowest value (146.33 cm) of this trait was occurred in strain 162 × spray (Table 3). The improvement of stem length may be attributed to the effect of the applied bacterium on the plant vegetative growth. Hernandez *et al.* [15] represented that the bacterial inoculation significantly increased maize plant height and stem diameter. Zahir *et al.* [16] also reported 8.5% enhancement in maize height as the result of *Azotobacter* and *Pseudomonas fluorescence* inoculation.

Panicle Length: Results indicated that strain significantly affected panicle length (p=0.01); the effect of application method and the interaction was not significant (Table 2). Among the strains, the highest value of panicle length was achieved in strain 87 (24.22 cm), which was 43.40% more than the control. The effect of the three application methods was not significantly different on this trait. Strain 87 × spray was the best treatment and increased panicle length by 73.29%, compared with the control (Table 3).

The obtained results are in agreement with those of Zahir *et al.* [16] who represented that the co-inoculation of *Azotobacter* and *Pseudomonas* increased maize grain yield by 19.8%. In another experiment, maize grain yield was, but ear length was not affected by the co-inoculation of *Azotobacter* and *Azospirillum* [17]. Hasanzadeh *et al.* [18] reported that phosphorus absorption facilitating bacteria significantly affected the number of kernels in barley panicle; increasing the number of kernels by 17% compared with the control.

The Number of Leaves: According to the analysis of variances, strain, application method and their interaction significantly affected this trait (Table 2). Among the different strains, strains 87 and 162 were the best treatments, without any significant differences (11.78 and 11.22 leaves in plant, respectively). Among the three application methods, the double inoculation was the most effective treatment on the number of leaves with 11.50

Table 2: Analysis of variances of the measured traits.

SOV	df	Mean Squares (MS) Stem length	Panicle length	Number of leaves	Stem dry weight	Fresh forage yield	Dry forage yield
Replication	2	ns	ns	*	ns	ns	ns
Pseudomonas strain	3	*	**	**	**	**	**
Application method	2	**	ns	**	**	**	**
Strain×Method	6	**	ns	*	*	**	**
Error	22	72.9	0.182	0.546	41.3	3.47	1.53
CV (%)	35	16.1	17.7	10.2	34.8	12.5	14.1

ns, nonsignificant; **, significant at P=0.01; *, significant at P=0.05.

Table 3: Effect of *Pseudomonas* strains, application methods and their interactions on the measured traits.

Treatments	Stem length (cm)	Panicle length (cm)	Number of leaves / plant	Stem dry weight (g / plant)	Fresh forage yield (ton/ha)	Dry forage yield (ton/ha)
No inoculation	153.11a	16.889d	9.89c	33.2c	32.26d	7.66d
Strain 87	165.67a	24.222a	11.78a	46.4a	46.61a	13.26a
Strain 162	162.00a	20.133b	11.22a	39.4b	42.37b	11.59b
Strain169	161.44a	18.033c	10.67b	36.5bc	39.81c	9.38c
Seed inoculation	159.08a	18.017a	11.08b	38.4b	40.31b	10.23b
Spray	154.67a	16.192a	9.83c	34.4c	36.47c	8.37c
Double inoculation	162.17a	20.00a	11.50a	42.6a	47.76a	14.30a
87×Seed	151.33fg	24.20a	11.67b	38.72d	46.35b	13.44b
87×Spray	149.33g	25.30a	10.67bc	33.48f	43.84c	12.52c
87×Double	164.33a	25.167a	12.00a	48.53a	48.10a	14.27a
162×Seed	148.00fg	21.933b	12.27a	34.97e	44.21c	10.95d
162×Spray	146.33g	19.267b	11.00b	33.26f	41.88d	9.37e
162×Double	163.67b	23.84b	12.67a	45.69b	46.78b	11.55cd
169×Seed	157.67def	17.50c	9.56c	35.21e	37.97e	8.24fg
169×Spray	152.00efg	17.333c	9.33c	30.54fg	38.36e	7.67g
169×Double	159.67c	15.767d	10.67bc	44.92b	42.67d	8.85ef

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$.

leaves compared with the control (11.08 leaves). Finally, among the interactions, the highest number of leaves was achieved in the double inoculation × strain 162 (12.62 leaves/ plant).

Hamidi and Asgharzadeh [19] found that inoculating maize seeds with *Azotobacter chrococcum*, *Azospirillum brasilense*, *A. lipoferum* and *Pseudomonas fluorescens* increased the number of leaves. Zahir *et al.* [16] also reported the enhancement of maize leaves number inoculated with *Azotobacter* and *P. fluorescens*. Other studies represented that the bacterial inoculation improved catalase activity and chlorophyll content, growth and the number of leaves in sunflower [20, 21]. Rohitashav-Singh *et al.* [22] reported the increased number of leaves in maize as the result of bacterial inoculation. They concluded that this improvement is resulted by the increased production of gibbereline, which increases cells elongation and auxin, which promotes cell division.

Stem Dry Weight: Analysis of variances revealed the significant effect of strain, application method and their interaction on stem dry weight (Table 2). Mean comparison indicated that strain 87 was the best bacterial strain (46.4 g) and the double inoculation was the best application method (42.6 g). The highest value of stem dry weight was obtained in the interaction of the double inoculation × strain 87, which was 39.98% higher than the control. These findings are supported by the past studies of Zahir *et al.* [16] who found that inoculating maize seeds with *Azotobacter* and *Pseudomonas* increased the shoot dry weight. Rusta *et al.* [23] also reported that *Azospirillum* inoculation increased maize stem dry weight.

Fresh Forage Yield: Bacterial strain, application method and their interaction significantly affected fresh forage yield ($p \leq 0.01$; Table 2). According to the mean comparison, strain 87 was the best strain and the double inoculation was the best application method with 46.61 and 47.76 ton/ha fresh forage yield, respectively. The highest fresh forage yield (48.10 ton/ha) was achieved in the interaction of the double inoculation × strain 87 (Table 3). These results are in agreement with those of Nanda *et al.* [24] who reported that inoculating maize seeds with *Azotobacter* and *Azospirillum* increased the forage yield. In another experiment, it was observed that application of *Azotobacter*, *Azospirillum* and *Pseudomonas* increased maize fresh plant weight by 33%.

Dry Forage Yield: Analysis of variances (Table 2) indicated that the treatments of this experiment and their interaction significantly affected dry forage yield ($p \leq 0.01$). Among the tested strains, strain 87 had the highest dry forage yield (13.256 ton/ha). Moreover, the double inoculation was the most effective application method and produced 14.30 ton/ha dry forage yield. The highest value of this trait was achieved in the interaction of the double inoculation × strain 87 which was 132.03% higher than the control (Table 3).

Hamidi and Asgharzadeh [19] conducted an experiment to evaluate the effect of *Azotobacter chrococcum*, *Azospirillum brasilense*, *A. lipoferum* and *Pseudomonas fluorescens* on maize growth and yield production. Their results indicated that forage yield was the highest when the four microorganisms were applied together and the efficiency of the individual application of microorganisms was higher in *Pseudomonas*, *Azospirillum* and *Azotobacter*, in order. In another experiment it was reported that application of PGPR increased maize shoot dry weight by 42.6% [25]. Hasanzadeh *et al.* [18] also found that bacterial inoculation increased dry matter accumulation in wheat and sorghum.

CONCLUSION

The overall results of this experiment represented that strain 87 was the best *Pseudomonas* strain tested in this experiment which increased dry forage yield by 73.11% compared with the control (no inoculation). Moreover, the double inoculation (seed inoculation + spray) was the most effective application method and increased dry forage yield by 39.79% and 70.85% compared with seed inoculation and spray, respectively.

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