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Research Article

Morphological and Phenological Responses of Sunflower to Nitrogen Fertilization and Plant Growth Promoting Rhizobacteria under Rainfed Conditions in Pakistan

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Abstract: Present study was conducted to evaluate the morphological and phenological responses of two sunflower (*Helianthus annuus L.*) cultivars (SFH-70 and RA-533) to nitrogen (N) fertilization and inoculation with plant growth promoting rhizobacterias (KS_{41} and KS_{42}) during 2015 at University of Poonch, Rawalakot, Azad Jammu and Kashmir. Treatments included un-amended control, KS_{41} (PGPR strain 1), KS_{42} (PGPR strain 2), UN (urea N), KS_{41} + UN and KS_{42} + UN. Nitrogen was applied at a rate of 150 kg N ha-1. The experiment was laid out in two factorial split-plot arrangement under randomized complete block design (RCBD) with three replications. Among treatments, PGPRs with UN illustrated maximum plant height, stem diameter, shoot and root dry matter of sunflower. Similarly, chlorophyll contents, head diameter, and number of days taken to flower initiation, completion and maturity were significantly affected by the combined application of UN with KS_{41} and KS_{42} compared to their sole application. When compared with control overall increase in morphological and phenological traits ranged between 27 to 65 %. Among cultivars, SFH-70 morphological and phenological traits responded more positively towards combined application of PGPR and UN and were significantly higher than RA-533. Results from this study indicated that combined application of PGPR and UN significantly enhanced most of the morphological and phenological traits of sunflower cultivars. However, further investigations are required to confirm the effect of PGPR application with different N sources, N rates and soil conditions

Keywords: Inorganic N, Plant-growth-promoting rhizobacteria strains, Growth, Sunflower

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop, cultivated throughout the world under diverse environmental conditions [1]. Nutritional qualities of sunflower oil i.e. high smoke point, linoleic acid and other polyunsaturated fatty acid make it an excellent edible vegetable oil [2]. Despite its importance, total area under sunflower cultivation and its production in Pakistan is far less compared to other crops and cannot meet country's edible oil demand. Therefore, edible oil is imported in Pakistan and its import is next to petroleum. The shortage in domestic edible oil production and consumption can be met by bringing more area under sunflower cultivation and by increasing its average yield per hectare.

Low average yield of sunflower is mainly attributed to improper nutrition and poor management practices [3]. Among nutrients, nitrogen (N) is considered the most important and limiting nutrient whose deficiency modifies several morpho-physiological parameters [4]. The use of N fertilizer to enhance crop production is a common practice and the impact of N supply on the performance of sunflower has been broadly characterized [5]. However, recovery of

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applied fertilizer N is often as low as 50%, and N losses from soil-plant system impose adverse environmental impacts i.e. ammonia (NH_3) emissions results in smog formation and disturbs ecosystems equilibrium after its redeposition [6]. Further, extensive use of N fertilizers cannot bring long-term productivity. Therefore, alternative nitrogen (N) management practices are required for increasing N use efficiency (NUE), environmental protection and sustainable agriculture.

Among several options, application of plant growth promoting rhizobacteria (PGPR) with N fertilizer is an important option for improving NUE and sustainable agriculture production [7]. Plant growth-promoting rhizobacteria (PGPR) are clusters of bacteria that colonize plant roots and enhance crop growth and yield by modulating different processes i.e. phytohormones production, phosphorus solubilization etc. [8, 9, 10]. Beneficial effects exerted by PGPR have been widely documented on many plant species including field crops, vegetables, ornamentals, and forest trees [11]. Numerous studies revealed that synthetic N fertilizers and PGPR positively enhanced crops growth and yield [12, 13, 14]. The findings of Akbari et al [15]; Herman et al [16]; Kloepper et al [11] and Zadeh et al [17] suggest that PGPR are able to increase sunflower growth and yield through the production of IAA, phosphate solubilization, and antagonistic effect towards pathogens. Therefore, application of PGPR has the potential of reducing N fertilizers and pesticides use and bring sustainability in sunflower cultivation.

Under the present increasing food demands and the quest for sustainable agriculture, plant growth promoting rhizobacteria can play an important role in fulfilling the requirements of the growing world. The present work was conducted to study the impact of nitrogen and PGPR application, either alone or in combination, on morphological and phenological characteristics of two sunflower cultivars under rainfed conditions of Rawalakot, Azad Jammu and Kashmir, Pakistan.

2. MATERIALS AND METHODS

2.1. Site Description

The present study was carried out at the Research Farm of the Department of Soil and Environmental sciences, Faculty of Agriculture, University of the Poonch, lower Shamsabad Campus, Rawalakot, Azad Jammu and Kashmir, Pakistan. The area lies between latitude of 33°.51ˈN and a longitude of 73°.45ˈE at an elevation of 1800-2000 m above sea level in the north-east of Pakistan under the foothills of the great Himalayas, Poonch division, Azad Jammu and Kashmir (AJK). The topography is mainly hilly and mountainous with valleys and stretches of plains. The climate of the region is sub-temperate. Mean daily maximum and minimum air temperatures ranged from 27 to 29 °C (June–July) and 1.0 to −3.5 °C (January–February) accompanied by severe cold and snowfall. The area has a temperate monsoon climate and the rainfall ranges from 1200 mm to 2000 mm with 45% of the total precipitation during June–September and 43% during January to April. Predominant soils in the area Inceptosols (Humic Lithic Eutrudepts) [18]. The dominant land use in the area is dryland farming (100% rainfed). The most common cultivation pattern involves a rotation of winter wheat (*Triticum aestivum* L.) with maize (*Zea mays* L.).

2.2. Experimental Details

The experiment was conducted during 2015-16. Before the start of the experiment, the area was cleared and soil samples were collected and analyzed for physical and chemical properties. Soil analysis showed that the soil was silt loam having pH, 7.25, EC of 0.41 dS m^{-1}, bulk density of 1.28 g cm^{-3}, organic matter, 0.80%, total N 870 ppm, available P 2.43 ppm and exchangeable K 74.38 ppm. Seed bed was prepared by ploughing soil two times followed by planking to break soil clods and divided into 36 sub plots of 4 m^2. The experiment was laid out in two factorial split-plot arrangement under randomized complete block design (RCBD) with three replications using sunflower cultivars i.e. SFH-70 and RA-533 in main plots and treatments i.e. T_1: Control; T_2: KS_{41} (PGPR strain 1); T_3: KS_{42} (PGPR strain 2); T_4: UN_{150}(Urea N @ 150 N ha^{-1}); T_5: KS_{41}+UN_{150} and T_6: KS_{42}+UN_{150} in sub-plots. The bacterial strains KS_{41} and KS_{42} were obtained from Soil Biology and Biochemistry Laboratory, Land Resources Research Program, National Agriculture Research Center (NARC), Islamabad. Before sowing, inoculation of sunflower seeds with PGPR was done by mixing the seeds with 20% sugar solution under shed to elude harmful effects

of sunshine on PGPR. Seeds were hand sown in each sub-plot by maintaining 40 cm row to row and 25 cm plant to plant distance. Urea was used as a source of N and applied to the corresponding sub-plots according to the experimental layout before sowing. Basal dose of phosphorus (single super phosphate) was applied at the rate of 90 kg P_2O_5 ha^{-1}.

2.3 Measurements

2.3.1. Morphological Parameters and Chlorophyll contents

Morphological traits like plant height, stem diameter, head diameter, root and shoot dry weight were recorded from five plants of central three rows of each sub-plot. Plant height was measured with the help of a meter rod at the maximum vegetative growth stage (R_4 stage) while stem diameter was measured with the help of vernier caliber, head diameter with measuring tape. Root and shoot dry weight was recorded on top load weighing balance at maturity. Chlorophyll content was estimated by the method described by Wintermans and Demots [19].

2.3.2. Phenological parameters

Phenological traits including number of days from sowing to flower initiation, flower completion and days to flower maturity were recorded from five plants of central three rows of each sub-plot. Days to flower initiation were measured when 5% of the flowering buds were opened while days to flower completion were measured when 90-95% of the buds were opened.

2.3.3. Statistical Analysis

Experimental data is presented as mean values of three replicates. All data was subjected to analysis of variance (ANOVA) and differences among treatments were further separated and compared using Least Significant Difference (LSD) test at 0.05 level of probability using MSTAT-C software [20].

3. RESULTS

3.1. Changes in the Morphological Characteristics

Seed inoculation with PGPR strains (KS_{41} and KS_{42}), application of urea N (UN) and their combination had a significant ($p \leq 0.05$) effect on plant height, stem diameter, root length, shoot and root dry weight for both sunflower cultivars (SFH-70 and RA-533). The greatest plant height (175.33 cm), stem diameter (7.05cm), shoot (202 g) and root dry weight (21 g) for sunflower cultivar SFH-370 was obtained for plants treated with PGPR strains in combination with UN (Table 1). Similarly, stem diameter (6.77cm), shoot (254 g) and root dry weight (24.67 g) for cultivar RA-533 was also obtained from treatments where UN was combined with PGPR strains (Table 1). On an average of

Table 1. Effect of PGPR, UN and their combined application on growth parameters of two sunflower cultivars grown under rainfed conditions

Treatments	SFH-70					RA-533				
	Plant height	Stem diameter	Root length	Shoot dry weight	Root dry weight	Plant height	Stem diameter	Root length	Shoot dry weight	Root dry weight
	cm			g $plant^{-1}$		cm			g $plant^{-1}$	
Control	133.55 f	2.83 d	8.30 c	106.66 c	6.33 d	134.70 c	3.55 d	17.00 b	115.00 c	9.30 d
KS41	143.37 e	3.13 d	10.21bc	191.67 a	9.66 c	135.33 c	4.13 cd	19.6 ab	174.3 b	13.6 bcd
KS42	155.60 d	4.15 c	11.36 b	109.00 d	12.00 b	139.43 b	4.43 bc	21.70 a	176.67 b	10.0 cd
UN	165.69 c	4.45 c	17.87 a	158.33 b	9.67 c	174.56 a	4.25 cd	22.30 a	188.33 b	15.33 bc
KS41+UN	175.33 a	4.95 b	16.31 a	202.67 a	12.33 b	175.67 a	4.93 b	21. 21a	241.00 a	17.66 b
KS42+UN	169.00 b	7.05 a	18.00 a	201.00 a	21.00 a	174.60 a	6.77 a	22.00 a	254.00 a	24.67a
LSD	**2.07**	**0.47**	**2.19**	**12.48**	**1.10**	**8.59**	**0.73**	**2.71**	**18.88**	**2.81**

SFH-70 and RA-533 = sunflower cultivars; Control = (without PGPR and UN; KS41 and KS42= PGPR strains; UN= urea nitrogen. Values are mean of three replicates. Means followed by same letter did not differ significantly from Least Significant Test at $p \leq 0.05$ significance.

Table 2. Growth parameters of two sunflower cultivars in response to PGRP and UN application under rainfed conditions

Treatments	Unit	Treatments						Cultivars	
	cm	Control	KS_{41}	KS_{42}	UN_{150}	$KS_{41}+UN_{150}$	$KS_{42}+UN_{150}$	SFH- 70	RA- 533
Plant height	cm	134.17 e	139.67 d	147.67 c	170.17 b	175.50 a	171.83 b	115.00 c	9.30 d
Stem diameter	cm	3.19 d	3.65 d	4.26 c	4.33 c	4.97 b	6.93 a	174.3 b	13.6 bcd
Root length	cm	12.67 c	15.00 b	16.50 b	19.83 a	18.83 a	20.00 a	176.67 b	10.0 cd
Shoot dry weight	g $plant^{-1}$	110.83 e	183.00 b	135.33 d	173.33 c	221.83 a	227.50 a	188.33 b	15.33 bc
Root dry weight	g $plant^{-1}$	7.83 e	11.67 cd	11.00 d	12.50 c	15.00 b	22.83 a	241.00 a	17.66 b

Control = (without PGPR and UN; KS_{41} and KS_{42}= PGPR strains; UN= urea nitrogen. SFH-70 and RA-533 = sunflower cultivars. Values are mean of three replicates. Means followed by same letter did not differ significantly from Least Significant Test at $p \leq 0.05$ significance.

Table 3. Effect of PGPR, UN and their combined application on phenological parameters of two sunflower cultivars grown under rainfed conditions

Treatments	SFH-70					RA-533				
	Days to flower initiation	Days to flower completion	Days to flower maturity	Head diameter	Chloro-phyII content	Days to flower initiation	Days to flower completion	Days to flower maturity	Head diameter	Chloro-phyII content
				cm					cm	
Control	67.66 e	88.33 d	105.66 e	15.67 d	14.82 d		89.67 d	104.66 f	15.63 b	14.65 e
KS_{41}	72.00 d	92.00 c	112.30 c	19.03 bc	16.61 c	71.66 c	93.00 c	109.33 e	16.43 b	16.25 d
KS_{42}	73.66 c	92.33 c	110.33 d	19.77 b	17.63 c	71.00 c	92.33 c	113.00 d	16.63 b	16.49 d
UN	74.33 c	96.33 b	115.40 b	17.97 c	19.58 b	74.33 b	97.67 b	114.33 c	16.41 b	19.69 c
KS_{41}+UN	78.59 a	98.33 a	117.29 a	23.47 a	21.75 a	73.64 b	97.33 b	116.33 b	23.25 a	27.68 a
KS_{42}+UN	77.69 b	97.33 ab	117.34a	23.50 a	21.32 a	76.66 a	98.67 a	118.33 a	24.73 a	21.88 b
LSD	**0.99**	**1.37**	**1.03**	**1.36**	**1.21**	**0.85**	**0.91**	**1.28**	**1.53**	**0.92**

SFH-70 and RA-533 = sunflower cultivars; Control = (without PGPR and UN; KS_{41} and KS_{42}= PGPR strains; UN= urea nitrogen. Values are mean of three replicates. Means followed by same letter did not differ significantly from Least Significant Test at $p \leq 0.05$ significance.

Table 4. Phenological response of two sunflower cultivars in response to PGRP and UN application under rainfed conditions

Treatments	Unit	Treatments						Cultivars	
		Control	KS_{41}	KS_{42}	UN_{150}	$KS_{41}+UN_{150}$	$KS_{42}+UN_{150}$	SFH- 70	RA- 533
Days to flower initiation		69.00 d	72.00 c	72.00 c	74.00 b	76.00 a	77.00 a	74.00 a	72.00 b
Days to flower completion		89.00 c	92.00 b	92.00 b	97.00 a	98.00 a	98.00 a	94.00 a	95.00 a
Days to flower maturity		105.0 e	111.0 d	112.0 d	115.0 c	117.0 b	118.00 a	113.00 a	113.0 b
Head diameter	cm	15.67 c	17.76 b	18.15 b	17.19 b	23.33 a	24.12 a	19.89 a	18.85 b
Chlorophyll content		14.75 e	16.43 d	17.09 d	19.60 c	24.70 a	21.63 b	19.44 a	18.62 b

Control = (without PGPR and UN; KS_{41} and KS_{42}= PGPR strains; UN= urea nitrogen. SFH-70 and RA-533 = sunflower cultivars. Values are mean of three replicates. Means followed by same letter did not differ significantly from Least Significant Test at $p \leq 0.05$ significance.

both cultivars, when compared with control, sole application of UN and PGPRs increased plant height, stem diameter, root length, shoot and root dry weight by ranging from 7 to 59 % (Table 2). However, when PGPRs were combined with UN, morphological parameters except root length showed maximum increase. Among both cultivars, SFH-70 performed significantly better than RA-533. On an average of both cultivars, significantly higher growth was observed where PGPR strains were combined with UN. Among cultivars, SFH-70 responded more positively toward PGPR and UN application compared to RA-533 in terms of plant height, stem diameter, root length, shoot and root dry weight (Table 2).

3.2. Changes in the Phenological Characteristics

PGPR inoculation and UN significantly affected phenological characteristics of both sunflower cultivars (Table 3). Maximum number of days to flower initiation, completion and maturity were observed in plants subjected to combined treatment of PGPR with UN. Similarly, among both cultivars, maximum head diameter and chlorophyll content were also observed with combined application of PGPR with UN compared to control and their sole application. On an average of both cultivars, combined application of PGPR and UN prolonged number of days to flower initiation, completion and maturity compared to their sole application (Table 4). Among both cultivars, similar to growth characteristics, SFH-70 performed significantly better than RA-533 for phenological characteristics except for days to flower maturity.

4. DISCUSSION

Nitrogen (urea N) and PGPR applied alone did not increase growth and phenological characters of two sunflower cultivars under rainfed conditions significantly compared to their combined application (UN + PGPR). Generally, N fertilization increases growth and yield of crops compared to unfertilized crops. However, additional increase over UN in growth and phenological characteristics of plants after addition of PGPR could be attributed to enhancing plant resistance to phytopathogens through several mechanisms [21]. These mechanisms include bioavailability of nutrients, counteracting biotic and abiotic stress, and production of volatile organic compounds and enzymes to combat disease [22]. Similar results for the combined application of UN with PGPR over their sole application were reported in previous studies [23]. Increased plant height and stem diameter in response to UN + PGPR application over UN treatment could be due to effect of PGPR on nutrient uptake or increases nutrient availability by nitrogen fixation, mineralization of organic compounds, solubilization of mineral nutrients, and production of phytohormones [24]. Soleimanzaden et al [25] reported that application of nitrogen along with *Azotobacter* not only enhance nitrogen uptake but also increase the uptake of other nutrients as well, by converting them in plant available forms. Our result are in accordance with those of Bano and Fatima [26] who found that PGPR affects plant growth by phytohormones production and higher nitrogen uptake [22]. Similarly, Badr et al [27] found increased stem diameter of sunflower after application of nitrogen and microbial inoculation.

Root length in UN + PGPR treatments was not significantly higher than their sole application. However, root length in UN + PGPR treatments demonstrated that there was some opportunity for soil microorganism to interact with root in the rhizosphere, which in turn, affects the root length. Spaepen et al [28] had already demonstrated that inoculation with indole acetic acid (IAA) producing SP245 leads to stimulation in early plant development and significant increases in dry-weight yield of plants and roots, the total root surface and root hair formation.

Increase in number of days to different growth stages could be due to increased vegetative growth. Our results confirm earlier findings reported by various researchers [29-34]. Similarly, Zubillaga et al [34] and Muralidharudu [35] found that increased nitrogen availability and consequent uptake resulted in a delay in flowering of sunflower. On contrary, Ali et al [36] also reported early flowering of sunflower under low application levels of nitrogen. Sadiq et al [37] also have observed a delay in maturity and flowering of sunflower after application of nitrogen and PGPR. Prolonged phenological stages due to PGPR application in present study was an indication of suitable conditions and time available for plant growth and development. *Azotobacter* and *Azospirillum* are among PGPR that enhance crop

growth conditions through several mechanisms especially growth hormones production and improving the efficiency of roots [38, 39].

The effect of cultivar on growth and phenological characteristics was significant. Maximum and minimum changes in morphological and phenological parameters were recorded for SFH-70 and RA-533 cultivars respectively. The differences in morphological and phenological parameters among cultivars could be due to diverse genotypic reactions of cultivars. Further, this effect depends on the PGPR strain and population, the combination of the used PGPR strains, and the genotype of the plant and environmental conditions [40, 41, 42]. The difference of the plant response to PGPR can be explained by the interactions between plant and bacterial isolates [43].

Overall evaluation of our data from present study suggesting that application of PGPR with UN, may reduce the use of urea fertilizers and can be used for sustainable production of sunflower under rainfed conditions. PGPR are widely used in integrated agriculture for their N-fixing, P-solubilizing, and phytohormone-producing benefits which can improve crops yield and plant growth [27]. Furthermore, improvement in growth and phenological characters of sunflower in UN + PGPR treatments from our study showed the potential of PGPRs for sunflower production.

5. CONCLUSION

This work showed that combined application of PGPR and UN under rainfed condition significantly increased growth and improved phenological characters of two sunflower cultivars under rainfed conditions. Application of PGPR with UN can therefore help to reduce amount of UN that is necessary to obtain maximum growth for sunflower production. So, it is highly recommended to apply PGPR in combination with the UN for sunflower crop. However, further studies should be carried out to evaluate the effect of PGPR with different rates of urea fertilizers to demonstrate the potential of PGPR as a key component of crop production.

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