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Exploiting the Potential of Indigenous Plant Growth Promoting Rhizobacteria (PGPR) for Improving Growth and Yield Attributes of Canola in Western Saudi Arabia

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***Abstract* – Arid regions like western Saudi Arabia are characterized environmental stresses like drought, heat and salinity. These stresses hamper crop yield by altering plant physiological processes. Therefore, it is necessary to mitigate effect of these stresses for sustainable crop production. Plant growth promoting rhizobacteria (PGPR) can boost crop production by minimizing the negative impact of stresses in arid regions. Initially, we isolated large numbers of indigenous strains to find a rhizobacterial strain adapted to harsh climatic conditions of Saudi Arabia. Then, after the screening and characterization, one promising strain was selected for field evaluation. A field experiment in split-plot design with four replications and randomized complete block design arrangement was conducted. Two levels of fertilizer (F_0= No fertilizer and F_1= recommended fertilizer) as main plot factor and two levels of PGPR ($PGPR_0$= without PGPR and $PGPR_1$= with PGPR) as sub-plot factor were used. Data regarding growth and yield attributes of canola was recorded and analyzed. It was observed that PGPR inoculation significantly improved grain yield and 1000 grain weight than un-inoculated control at both levels of fertilizer. It can be concluded that PGPR with recommended dose of fertilizer can boost crop production under stressful climatic conditions of arid regions.**

***Keywords* – Canola, Plant Growth Promoting Rhizobacteria, Growth, Yield.**

I. Introduction

Optimum crop production to ensure food security on sustainable basis in arid regions like western Saudi Arabia is very challenging due to stressful environmental features of these tracts. These areas are characterized with high temperature and low rainfall which induce various natural environmental stresses like drought stress, heat stress and salinity stress. These stresses are very notorious in hampering crop yield by deteriorating soil quality and altering plant physiological processes. Prevailing scenario put great stress on agricultural scientists to find economically viable and environmentally sustainable ways for the mitigation of effects of environmental stress to boost plant growth and crop production. Recently, plant growth promoting rhizobacteria (PGPR) have gained much attention in this regard. Because they help plant fight with these environmental stresses through various mechanisms. PGPR is a diverse group of beneficial bacteria that inhabit the rhizosphere (thin layer of soil immediately surrounding plant roots) and promote the plant growth by various direct and indirect mechanisms [1,2]. They play a significant role both under stressed and normal conditions for improving plant growth and developmental processes [3,4,5]. The mechanisms that promote plant growth comprises nitrogen fixation, phosphorus solubilization, production of siderophores, plant growth regulators and organic acids as well as protection by enzymes like ACC-deaminase, chitinase and glucanase [6,7,8]. Altering vital plant physiological processes by bacterially produced plant growth regulators and hormones, enhanced nutrient availability by improved uptake of nutrients and nutrient solubilization, minimization of negative impacts of ethylene produced in response of various stresses, excretion of exopolysaccharides are some important mechanisms through which PGPR boost crop production [9,10,11,12,13].

There was previously no study on the effects of PGPR on crops in this area. Therefore, promising attributes of PGPR for sustaining crop production in stressful environments inspired us to utilize these natural resources to improve crop yield by mitigating negative impacts of environmental stresses. Although PGPR due to having ubiquitous nature are found everywhere but there natural population in soils is generally low. To reap maximum benefits from PGPR their population in soil must be in optimum number for effective functioning. Therefore, we hypothesized that increasing the population of PGPR by the addition of artificially multiplied indigenous PGPR in the laboratory can be helpful to promote crop production. This study was planned with the following objectives:

- To find the indigenous PGPR strain adaptable to harsh environmental conditions and having the ability to survive and proliferate in local soil conditions.
- To increase the population of selected strain in laboratory by providing optimum growth conditions.
- To evaluate the effectiveness of locally isolated and artificially multiplied PGPR strain for improving growth and yield attributes of canola under field conditions.

II. Materials and Methods

Isolation and Purification of Rhizospheric Bacterial Strains

To obtain PGPR strain adaptable to local environmental conditions isolation of bacteria was done from rhizosphere soil (soil in immediate contact with roots) taken from Hada al Sham (western Saudi Arabia) research station of King Abdulaziz University. To obtain PGPR strain adaptable to local environmental conditions isolation of bacteria was done from rhizosphere soil (soil in immediate contact with roots) taken from Hada al Sham (western Saudi Arabia) research station of King Abdulaziz University. Initially, 20 bacterial strains were isolated from the rhizosphere soil of different plant on Luria Bertani (LB) agar medium following the protocol adopted by Baig and his colleagues [14]. For this purpose plants were uprooted and bulk of non rhizosphere soil was removed by gentle shaking leaving behind only rhizosphere soil (soil strongly adhere to roots). After that these plants along with rhizosphere soil were kept in polythene bags and transferred to laboratory. In the laboratory plant roots were dipped in sterile saline to obtain soil suspension. A loopful suspension from each sample was streaked on LB agar plates and plates were incubated at 28 °C for 72 hours. After that morphologically different colonies showing prolific growth were isolated and purified by serial re-streaking on fresh agar plates every time.

Screening of PGPR on the Basis of their Plant Growth Promoting Potential

To find out most promising strain among the 20 pre-isolated bacterial strains a jar screening trial in growth chamber with three replications of each strain using peat moss as growth medium was conducted. Broth culture of each strain in tryptic soy broth (TSB), a general purpose medium [15] was prepared. Single colony of each strain was transferred to conical flask containing 250 ml sterilized nutrient broth and flasks were put in shaking incubator (120 rpm) at 28 ^{o}C for 7 days. Seeds of canola (cv. UAF-11) were surface sterilized by first dipping in 95% ethanol for short time and then dipping in 0.2% $HgCl_2$ solution for 3 minutes. The seeds then were washed with sterile distilled water and dipped in sterilized water to imbibe. Then imbibed seeds were dipped in broth culture for 10 minutes to inoculate with PGPR. Control seeds were treated with sterilized TSB solution. Then inoculated seeds were allowed to grow in jiffy-7 plates (peat moss medium) for 3 weeks. Sterile Hoagland nutrient solution was used to irrigate the seedlings. Plates were placed in growth chamber and after 3 weeks shoot length, shoot fresh weight (g/plant), root length and root fresh weight (g/plant) were recorded (data not showed) and one most promising bacterial strain was selected on the basis of its positive effect on above mentioned plant growth parameters.

Characterization of Selected PGPR Strain

To confirm the plant growth promoting activities of the most promising strain identified in screening trial following biochemical characterizations were carried out (Table-1).

Indole Acetic Acid (IAA) Production

IAA production potential of selected strain was assessed by following the spectrophotometric measurement protocol adopted from Patten, University of New Brunswick, Canada [16,17].

Phosphate solubilization

Quantitative determination of phosphate solubilization activity was determined by phosphomolybdate blue color method using Pikovaskya media [19] that was prepared following procedures described by Nautiyal [20]. Concentration of P in the media was determined by spectrophotometer.

ACC-Deaminase Activity

Modified method of Honma and Shimomura [21] was employed to assess the quantitative ACC deaminase activity of selected strains. Amount of α-ketobutyrate produced by the breakdown of ACC (precursor of α-ketobutyrate) through the action of bacterial ACC-deaminase of selected strains was measured by comparing the absorbance of sample with standard curve drawn by taking the absorbance of standards (0.1 to 1.0 µmol) of pure α-ketobutyrate at 540 nm.

Siderophore Production

The selected PGPR strain was also assessed for siderophore production using chrome azurole S (CAS) agar plates. CAS agar plates were prepared following the exact procedure described by Louden et al. [22]. The plates were spot inoculated with the PGPR isolate as described by Clark and Bavoil [23] for bacteria and were kept in an incubator at 30 °C for 5 days. The bacteria developed around a yellow–orange halo which indicated that this strain has potential for siderophore production.

Field Evaluation of PGPR for their Plant Growth Promoting Potential

To test the effectiveness of PGPR under field conditions for improving plant growth and yield a field experiment using canola as test crop was conducted at Hada al Sham research station of King Abdulaziz University-Jeddah. Experiment was planned according to split -plot design in randomized complete block design arrangement with four replications. The field soil was sandy loam in texture, having pH, 8.2; EC, 2.8 dS m^{-1}; 0.65% of organic carbon;

0.038% N, 7.3 and 197 ppm of available P and K, respectively. Two levels of fertilizer (F_0= No fertilizer and F_1= recommended fertilizer) as main plot factor and two levels of PGPR ($PGPR_0$= without PGPR and $PGPR_1$= with PGPR) as sub-plot factor were used. PGPR was applied as seed inoculation. Firstly, the experimental field was prepared using rotary plow. After that main factors were applied and sub plots of 2.5 x 1.5 m^2 were prepared. Then for seed inoculation seeds of canola (cv. UAF-11) were surface sterilized by first dipping in 95% ethanol for short time and then dipping in 0.2% $HgCl_2$ solution for 3 minutes. Surface disinfected seeds were then inoculated with the broth mixed with 10% sugar solution, peat and clay (Kaolin) mixture (peat to clay ratio, 1:1 w/w). The seeds were shaken well until a fine coating appeared on seeds. Control seeds were treated with sterilized peat plus clay mixed with sterilized broth medium (without bacterial cells) and sugar solution. Inoculated seeds were placed overnight for drying under laboratory conditions at 26 °C. Sowing was done with row to row and plant to plant distance of 40 and 30 cm respectively. Other cultural practices were consistent with local (farmers' practices in Hada Al Sham) agronomic practices.

Collection and Statistical Analysis of Data

To evaluate the effect of the treatments on crop growth and yield data regarding plant height (cm), number of pods $plant^{-1}$, number of grains pod^{-1}, grain yield (t/ha) and 1000 seed weight (g) was recorded and statistically analyzed (Table-1). All data were recorded according to standard procedures [24,25,26]. Before harvesting, 10 random guarded plants in each sub-sub plot were tagged to record data regarding plant height (cm) number of pods plant-1, number of grains pod-1. For grain yield an area of 1 m2 in the center of each sub-sub plot (to avoid marginal effect) was harvested. Data recorded for each trait was separately exposed to the analysis of variance (ANOVA) to check significance among treatments using Statistic 8.1 computer software and means were then compared using least significant difference (LSD) test according to Steel & Torrie [27].

III. RESULTS AND DISCUSSION

The results of effect of PGPR with and without fertilizer on growth and yield attributes of canola are presented in table. It is clearly indicated that fertilizer has significant effect on all the parameters studied. On the other hand PGPR and interaction between PGPR and fertilizer showed significant influence on grain yield and 1000 grain weight only.

Plant Height

Regarding plant height maximum plant height (110.38 cm) was observed under fertilizer treatment that is significantly different from without fertilizer treatment (88.32 cm). Although PGPR inoculation did not showed significant effect on plant height but inoculated treatment has more plant height than uninoculated one. The results of this study confirmed the findings of other researchers [28,29,30,31] who also reported improvement in plant height with the application of PGPR.

Number of Pods per Plant and Number of Grains per Pod

As for as number of pods per plant and number of grains per pod are concerned, although PGPR has not significantly improved these parameters however, mean comparison showed more number of pods per plant and more higher number of grains per pod. These results are contradictory to some other researchers [25,29,32,33,34] who reported significant improvement in these parameters with PGPR application. Fertilizer treatment proved statistically superior over unfertilized treatment in improving number of pods per plant and number of grains per pod. Highest number of pod per plant (162) and highest number of grains per pod (26) were observed under fertilizer conditions. These findings are also confirmed by [25] who reported significant increase in these parameters with increasing amount of fertilizer. Significant improvement in these parameters by the use of fertilizer has also been reported by [35,36].

Grain Yield and 1000 Grain Weight

In case of grain yield (kg/ha) and 1000 grain (g) weight PGPR application and fertilizer treatment are statistically superior to without PGPR and unfertilized treatment. Here, interaction between PGPR and fertilizer was also significant. Mean comparison clearly demonstrated that PGPR application along with recommended fertilizer produced significantly higher grain yield (509 kg/ha) than PGPR without fertilizer (403.75 kg/ha). Similarly, PGPR under no fertilizer have produced more grain yield (302.25 kg/ha) than without PGPR (280 kg/ha). Regarding 1000 grain weight, mean comparison of interaction between PGPR and fertilizer has also proved that PGPR application produced heavier grains than without PGPR both under fertilized and unfertilized conditions. The results of this study regarding the effect of PGPR and fertilizer on grain yield and 1000 grain weight are also in line with other researchers [37,30,38] who also document significant enhancement in these parameters by the interaction of PGPR and fertilizer.

Table 1. Biochemical characterization of selected PGPR strain

PGPR Strain	**IAA production ($\mu g\ mL^{-1}$)**		**P solubilization ($mg\ mL^{-1}$)**		**ACC-deaminase activity (μM α-ketobutyrate produced)**	**Siderophore production**
	With tryptophan	Without tryptophan	P in medium	pH of Medium		
SH-17	4.32	1.74	29.75	5.91	1.11	+

Table 2. Effect of PGPR inoculation with and without fertilizer on growth and yield attributes of canola.

Treatments	PH (cm)	NOP (per plant)	NOG (per pod)	GY (kg/ha)	1000-GW (g)
Fertilizer					
F_0 (no fertilizer)	88.32[b]	118.25[b]	19.500[b]	291.13[b]	2.14[b]
F_1 (with fertilizer)	110.38[a]	162.25[a]	25.750[a]	456.38[a]	2.68[a]
PGPR					
P_0 (no PGPR)	98.21[a]	135.88[a]	22.125[a]	341.88[b]	1.90[b]
P_1 (PGPR)	100.49[a]	144.63[a]	23.125[a]	405.62[a]	2.51[a]
Significance					
Fertilizer	**	**	**	**	**
PGPR	ns	ns	ns	**	**
Fertilizer * PGPR	ns	ns	ns	*	*

PH: Plant Height, NOP: Number of Pods, NOG: Number of Grains, 1000-GW: 1000 Grains Weight, GY: Grain Yield. Means followed by the same letters in each column and treatment showed no significant difference by LSD (P = 0.05). *, ** and ns showed significant difference at 0.05, 0.01 probability levels and not significant, respectively.

IV. Conclusion

In general, the results of this study implied that application of PGPR along with recommended dose of fertilizer can improve growth and yield attributes of canola in natural stressful environment of arid climatic conditions. The findings of study are inspiring to continue the use of PGPR for use in arid area to improve overall crop production for sustainable food security.

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