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Evaluation of Efficiency of Two Local Rhizobium Leguminosarum Isolates on Root Nodulation and Growth of Faba Bean (*Vicia faba L.*)

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A B S T R A C T

This study was conducted at the Libyan Biotechnology Research Center to investigate and evaluate the efficiency of two local isolates of Rhizobium Leguminosarum bacteria in root nodulation and improvement of the growth of faba bean. The two studied isolates were isolated from two geographically different regions in Libya (Qasir Ibn Ghashir and Shahhat). The experiment was designed according to the completely randomized Design (CRD). The experiment included two treatments (isolates) and three replicants of every isolate. The results showed superiority of R2 isolate in all studied growth characteristics (plant height, number of leaves, root length, root fresh weight, and the fresh and dry weight of stems and leaves) when compared to R1 isolate. Treating the plant with R2 isolate increased also the number of active nodules and the total weight of nodules. The results of all examined characteristics indicate that R2 isolate which is isolated from shahhat is significantly better in improving plant growth, which is as a result of the effect on nitrogen fixation and production of some plant hormones.

1 Introduction

Faba Bean (*Vicia faba L.*) is considered one of the oldest crops cultivated all over the world (Mínguez and Rubiales, 2021). The Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe, and North Africa are the major producers of faba beans (Rahate *et al*,. 2020). Among the more than 50 bean producing countries, about 90% of production is in Asia, the European Union and the African region (FAO, 2020).

Vicia faba is one of the winter crops belonging to the legume family, whose beans contain high percentage of protein, about 25 - 40% (Natalia *et al*., 2008). In addition, the beans contain carbohydrates that may zreach 56% in most varieties, and this increases the importance of the crop due to its high nutritional value. In addition, one of the faba bean cultivation benefits is the improvement of soil properties, through the symbiotically fixation of atmospheric nitrogen by Rhizobium (Zaki *et al*., 1997), therefore, Faba bean plants are used in agricultural rotations for improving soil properties (Carmen *et al*., 2005). Faba Bean is adapted to a wide range of soil pH 4.5-8.3, which resulted in yield increasing. Dry beans are also used as an animal fodders to improve the productivity. Faba bean is an important crop in regard to the environmental, nutritional and economic point of view (Xiao *et al*., 2021).

Conversely, Libyan soils are inefficient in nitrogen. Bio-fixed nitrogen through the symbiotic relationship between legumes and Rhizobium as a source of nitrogen supply is of great importance (Martin,1982), and therefore Rhizobium plays a major role in agriculture by providing soils with a part of the nitrogen fixed from the atmosphere (Verma and long ,1983). The biological

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fixation of atmospheric nitrogen represented by the symbiotic relationship between Rhizobium and leguminous plants requires sufficient Rhizobium number in the soil. Treating the legume seeds with a specialized Rhizobium species before sowing has economic benefits, as it is considered an effective way to increase soil fertility (Hardarson, 1993).

In a field study, the results showed there was a significant effect of inoculation with root nodulation bacteria, which led to proportion increasingof protein, carbohydrates, and fiber in seeds (Abbas and Majid ,2006). Testing of 12 Rhizobium strains isolated from Tigre highlands in northern Ethiopia showed that there were significant differences in the number of bacterial nodules, fresh weight, plant length, and nodule color variation (Alemayehu, 2009). Turki, 2011 also found that inoculated faba bean plants by Rhizobium were better than un-inoculated ones in regards to nodule numbers, nodule weights, shoot, nitrogen concentration, number of branches, shoot dry weight. Inoculation faba bean with rhizobium increased significantly the yield, seed moisture, ash, raw fibers and raw protein (Rugheim *et al*., 2012). It was found by Osama et al., 2021 that inoculation by Rhizobium led to a significant increase in nodule numbers, shoot and root growth, and the nitrogen content in plant and soil.

The aim of this study was to obtain Rhizobium strains that are highly efficient in nitrogen fixation to use them as a bio-fertilizer which is an alternative to mineral nitrogen fertilizers that has negative effects one human and environment.

2 Materials and Methods

This study was conducted at the Libyan Biotechnology Research Center using faba bean plants and the bacterial species Rhizobium Leguminosarum.

2.1 Bacteria Used in the Study

Two isolates of the species Rhizobium Leguminosarum were isolated from two geographically different regions of Libya (Qasir Ibn Ghashir and Shahhat) at the Libyan Biotechnology Research Center according to the known isolation procedures used to get pure isolates by using the medium of yeast extract mannitol agar (table 01). The isolates were observed in a fridge in agar slant tubes until using them in the field experiment.

Table 1: Composition of yeast extract mannitol agar

Parameters	Value
Yeast Extract	0.5g
Mannitol	10g
$MgSO_4.7H_2O$	0.2g
K_2HPO_4	0.5g
NaOH	0.1g
Agar	15g

Bacterial inoculums of the two isolates (R1: Qasir Ibn Ghashir isolate, R2: Shahhat isolate) were prepared in a yeast extract mannitol solution. The used tools, distilled water and the medium were sterilized by Autoclave AUX-512-020D by 120C° and a pressure of 15 psi for 20 minutes (Zaki, 1997). The inoculated media were incubated using a shaker incubator (Shaking Incubator 3023-3033 GFL) by 28C° for 72 hours.

2.2 Characteristics of Used Soil and Irrigation Water

The soil properties mentioned in tab. 02 were determined according to the known procedures of soil analysis. Calcium carbonate was estimated by calciminer. The Jackson method, 1973 was used to estimate the available phosphorus in the soil. The total soil nitrogen was estimated according to Kjeldahl method, while the zinc concentration was according to DTPA method. For estimation of available potassium was used a flam photometer.

Table 2: Some properties of used soil

parameters	Value
Physical properties	
Sand	77.14%
Silt	8.18%
clay	14.68%
Soil texture	sandy loamy
Chemical properties	
Organic matter	0.33%
Calcium carbonate	5.0%
Available zinc	0.26 ppm
Total nitrogen	0.25%
available phosphorous	0.6 ppm
available potassium	97 ppm
pH	7.95
EC	1.04 dS/m

Irrigation water samples were taken in a sterile bottles to determine the mentioned parameters in table3.

Table 3: Chemical properties of irrigation water

parameters	Value
pH	6.8
EC	1.85 dS/m
TDS	1184 ppm
K^+	39.6 ppm
Na^+	105 ppm
Ca^{+2}	58 ppm
NO_3	10.81 ppm

2.3 Seed Inoculation and Planting

Faba beans of the cultivar Nbella Mora obtained from an agricultural store were sterilized using a sodium hypochlorite solution and ethanol 95%. Then, the seeds were treated with the isolated Rhizobium isolates after by soaking them in a solution of adhesive substance (sugar).

The inoculated beans were planted in polyethylene bags filled with 5 kg of a sieved soil. The experiment was designed according to the completely randomized Design (CRD). Two seeds were sowed in every bag of three bags (replicants) for every isolate (treatments R1 and R2). The soil was fertilized according to the crop requirements.

The plants were harvested after 49 days of sowing, the, the roots were separated from the shoots. comparison tests related to the vegetative growth, root growth and nodulation were done to compare between the two treatments.

Statistical Analysis

The statistical analysis were performed according to the Snedecor and Cochran method 1980. For the comparison of averages between treatments, the method of least significant difference (LSD) at 0.05 significant level using the SAS program 1999 were used.

2.4 Results and Discussion

2.4.1 Effect of Rhizobium Isolate on the Shoot and Root System

The results mentioned in Table 4 indicate that the two bacterial isolates are different in effect when compared the results of shoot and root system. According to these results, it's clear that R2 isolate is significantly superior in plant length, by which the plants were 7.06 cm taller than the plants treated with isolate R1, Also the root length, number of leaves and the fresh weight of the root system increased significantly in R2 treatment, by which the values were higher by 7.21 cm in the root length, 4.43 in the number of leaves and by 3.10 g in the fresh weight of the root system. Such increase in the studied attributes were also mentioned by Al-Tamimi 1998, Al-Baldawi 2004 and Saad 2011, which indicates that the strain is an affective factor in nitrogen fixation that enhances the plant nutrition.

The reason beyond the enhanced growth of the root system by R2 Isolate can be explained as a result of production of some plant metabolites such as Riboflavin, Cytokinine and Gibberellin which play an important role in root growth, and that improves the plant growth due to the increased uptake of nutritional elements (Dakora, 2003). R2 isolate as shown in table 05 lead also to a significant improvement in both wet and dry weights of both stems and leaves. That corresponds with the results obtained by Alemayehn, 2009 and Rugheim *et al*., 2012 in their studies of the effect of different Rhizobium strains on nitrogen fixation. The increased weight of the plants is an indicator of increased nitrogen fixation ratios (Somasegaran and Hoben, 1994). As nitrogen is the most important element in plant nutrition, this explains that these differences between the both isolates are due to different efficiencies of nitrogen fixation, by which the atmospheric nitrogen is converted into available form to the plant which is used to create amino acids and proteins that lead to improvement of the plant growth.

Table 4: Effect of Rhizobium Isolate on the shoot and root system

Treatment	Plant height (cm)	Leave number	Root height (cm)	root Fresh weight (g)
R1	52.14b	23.97b	11.47 b	17.33 b
R2	59.20a	31.18a	15.9 a	20.43 a
LSD 0.05	3.17	2.29	0.25	0.06

Table 5: Effect of Rhizobium Isolate on the fresh and dry weights

Treatment	Stem Fresh weight (g)	leaf Fresh weight (g)	Stem dry weight (g)	leaf dry weight (g)
R1	12.79 b	6.92 b	1.14 b	1.85 b
R2	14.41 a	8.63 a	1.35 a	2.05 a
LSD 0.05	0.01	0	0.008	0.01

2.4.2 Effect of Rhizobium Isolate on the Nodulation of Faba Bean

The results mentioned in table 6 showed significant increasing in the number of effective nodules and also the total number and weight of nodules produced by the R2 isolate. Such increasing in weight and numbers corresponds with the results obtained by Alemayehn, 2009 and Saad, 2011. This explains that the increasing in nitrogen fixation by R2 isolate mentioned previously is related to the better activity of R2 isolate in root penetration, nodule formation and to the difference in reproduction and generation time between the two studied isolates.

Table 6: Effect of Rhizobium Isolate on the nodulation of fava bean.

Treatment	Total number of nodules	Number of active nodules	Ratio of effective nodules %	Nodule total weight (mg/plan)
R1	28.4 b	54.3 b	65.61 b	85.73 b
R2	34.2 a	66.8 a	66.10 a	133.68 a
LSD 0.05	0.14	0.56	0.41	0.70

3 Conclusion

This study showed significant results in all studied attributes, which indicates that the Rhizobium strain is an affective factor in nitrogen fixation, and as a result of this, R2 isolate which is isolated from Shahhat in eastern part of Libya was significantly more efficient in nitrogen fixation than the one isolated from Qasir Ibn Ghashir. That was shown as an enhancement of shoot and root system which is related to increasing in attributes connected to the nodule formation (nodule number, nodule weight and number of effective nodules). The different behavior of the two isolates can be explained as a result of an adaptation to the different environments.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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