Efficiency of Biological Nitrogen Fixation of 
Bradyrhizobium under Different Soil Fertility Levels as Measured by $^{15}$N Dilution Analysis

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Abstract: The objective of this study was to determine the efficiency of biological nitrogen fixation (BNF) of local Bradyrhizobium isolates in soil of various fertility levels using $^{15}$N dilution technique. Local isolates were obtained from cowpea rhizosphere in fields of different Iraqi provinces. Six isolates were selected in this study, which was conducted as a pot experiment under greenhouse conditions. Effects of the following fertility levels were evaluated: at F1, 0 mg N, P and K was added; at F2, 25 mg N/kg soil, 10 mg P/kg soil and 25 mg K/kg soil were added, respectively; the other two levels were F3 at which 50, 20 and 50 mg/kg soil and at F4 75, 30 and 75 mg/kg soil for N, P and K, respectively, were added. Urea, labeled with $^{15}$N 10% access atom (aa), was used as a source of N. The highest BNF was observed under the lowest fertility level, i.e., F1. BNF across all isolates was markedly decreased with the increase of nutrient application to soil, being totally eliminated at the highest fertility level F4. Numbers of nodules per plant root of all isolates were the least under the zero nutrients application and the highest nodules number were found under the highest levels of N, P and K application. Number of nodules does not necessarily reflect the best BNF efficiency of all isolates. However, fertility levels were of significant effect on average nodule number of all isolates. The lowest plant dry weight was under the first fertility level F1 irrelevant of Rhizobium isolates. In general, the highest plant dry weight was under the second soil fertility level F2.

Key words: Rhizobium, local Iraqi strains, cowpea, nodules number, dry weight.

1. Introduction

Deficiency in mineral N usually limits plant growth. Consequently, symbiotic relationships have evolved between plants and a variety of nitrogen-fixing organisms [1]. Successful Rhizobium-legume symbioses will definitely increase the incorporation of biological nitrogen fixation (BNF) into soil ecosystems. It had been stated by Tate [2] that Rhizobium-legume symbioses are the primary source of fixed nitrogen in land based systems and can provide well over half of the biological source of fixed nitrogen. Atmospheric N$_2$ fixed symbiotically by the association between Rhizobium species and legumes represents a renewable source of N for agriculture [3].

Therefore, BNF is an efficient source of N to plant and soil [3]. According to Paul [4] and Burns and Hardy [5], total annual terrestrial inputs of N from BNF was given by range from 139 million to 175 million tons of N. Symbiotic associations growing in arable land is accounting for 25%-30% (35 million to 44 million tons of N). Permanent pasture, however, is accounting for another 30% (45 million tons of N) [6].

Soil degradation is the most common problem worldwide, and it is important to stop the intensive depletion of soils by inconvenient agricultural practices. The vital process of BNF can play a key role in land remediation. An examination of the history of BNF shows that interest generally has focused on the symbiotic system of leguminous plants and rhizobia, because these associations have the...
greatest quantitative impact on the nitrogen cycle. A tremendous potential for contribution of fixed nitrogen to soil ecosystems exists among legumes [2, 3]. This enormous quantity will have to be augmented as the world’s population increases and as the natural resources that supply fertilizer-N diminish. This objective will be achieved through the development of superior legume varieties, improvements in agronomic practice and increased efficiency of the nitrogen-fixing process itself by better management of the symbiotic relationship between plants and bacteria. The symbioses between Rhizobium or Bradyrhizobium and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume based crop and pasture production than the application of fertilizer-N. The introduction of legumes into these pastures is seen as the best strategy to improve nitrogen nutrition of the grasses.

The above overview clearly indicates the significance of Rhizobium-legume symbioses as major contributors to natural or biological N₂ fixation.

Cowpea (Vigna unguiculata L.) as a legume crop was used as a test crop in this study because of its high adaptability to severe climatic conditions, like those prevailing in Iraq. It is a typical legume crop for the arid regions, of which Iraq is a part [7]. Furthermore, cowpea is a considerably important protein source for the low average income population in Iraq and in the most parts of the arid regions of the Middle East.

Therefore, the current investigation was conducted to evaluate the effect of the rate of fertilizer application on BNF and the most appropriate rate of fertilizers application that leads to the most efficient BNF process.

2. Materials and Methods

2.1 Collection of Nodules

Cowpea root nodules were collected from 20 locations from different field sites in 10 governorates in Iraq, belonging to the most important cowpea production area in Iraq (Basrah, Dhi-Qar, Misan, Wasit, Babil, Al Anbar, Baghdad, Salahudein, Suleimanyah and Ninevah).

Effective and healthy cowpea root nodules were collected from young, healthy and green cowpea plants of 45-60 days old. Cowpea plants in the sites were simply excavated from cultivated field and adhering soil particles were carefully removed. The roots were found to have nodules of various size and stage of development, of which reddish to pinkish healthy nodules of 0.3-0.6 mm diameter were excised from the root along with 0.5-1.0 mm root part on the both sides of nodule attachment. Excised nodules were washed with tap water, shade dried for 1-2 h and collected in nodule collection vials. The vials were labeled and stored at 4 ± 1 °C for short-term storage.

2.2 Bacterial Isolates

Isolation of cowpea Rhizobium colonies and maintenance of pure culture and authentication of isolates by infection tests were all done as mentioned in Ref. [8]. Furthermore, growth on yeast manitol agar-bromothymol blue (YMA-BTB) reaction was also done after [8].

2.3 Efficiency of the Rhizobium Isolates

This pot experiment was conducted as in Ref. [8]. Efficiency of BNF of the six isolates and control were evaluated in medium texture soils with three levels of fertility. Levels of fertility were: fertility level 1 (F1) = 0 mg N, P, K added; fertility level 2 (F2) = 25, 10, 25 mg/kg soil for N, P, and K, respectively, was added to soil; fertility level 3 (F3) = 50, 20, 50 mg/kg soil for N, P and K, respectively, were added; fertility level 4 (F4) = 75, 30, 75 mg/kg soil for N, P and K, respectively, were added.

Amount of N added at all rates of N application was labeled with \(^{15}\)N 10% access atom (aa). Soil, pots, seeds, planting and watering were based on the methodology as described in Ref. [8].
3. Results and Discussion

3.1 Effect of Soil Fertility Levels on BNF

Low nutrients availability in soils is one of the most limiting factors to productivity of cowpea. P and N are the most important elements for cowpea productivity [9]. Fig. 1 shows the BNF of the six isolates under four different levels of soil fertility.

Results showed that the highest BNF of all isolates was observed under the first fertility level, at which zero plant essential elements were added to the soil. Results showed that increasing soil fertility markedly reduced the BNF. This result is in agreement with that of Bantilan and Johasen [10], who showed that high N content in soil reduced the efficiency of BNF of nitrogen fixing bacteria. At the same time, low levels of nitrogen in soils which limit plant growth, also affect bacterial efficiency in BNF, which was also indicated by Weisany et al. [11]. Therefore, plant growth must be good enough to excrete the flavonoids, which help nitrogen fixing bacteria to infect root hair. Consequently, formulate the nodules and extend the bacteroid essential to fix N that can be utilized by the plant [12].

At N levels of 50 mg/kg and 75 mg/kg soil, BNF is totally eliminated in all types of isolates used in this study. Therefore, these results may suggest the need to determine the critical level of N in soils at which BNF is at the maximum. This will tremendously reduce the level of N fertilizer additions. This may also indicate the importance of BNF in crop production. It is well known that reducing application of N fertilizer reduces eutrophication and nitrogen contamination of water resources.

According to statistical analysis of data, the LSD for BNF% under fertility levels was 0.7698; LSD for BNF% of six isolates under fertility levels was 1.0184; LSD for BNF% as affected by isolate-fertility interaction was 2.0261. It showed that there is significant difference among isolates in their ability to fix N at various soil fertility levels. Also, there is significant difference among isolates in their ability to fix N.

3.2 Nodules Number as Affected by Soil Fertility Levels

Fig. 2 shows the number of nodules of the six isolates as affected by the fertility level of the soil. The number of nodules was clearly lowest under low soil fertility level. Isolates 3 and 47 formulate the

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![Fig. 1 Effect of soil fertility levels on BNF efficiency of local \textit{Bradyrhizobium} isolates.](image-url)
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Fig. 2  Number of nodules of *Bradyrhizobium* local strains on cowpea root as affected by soil fertility levels indicated.

highest number of nodules. All other isolates are relatively formulating equal number of nodules per plant.

Increasing the fertility to F2 resulted in considerable increase in number of nodules of isolate 26, 3 and 9. The greatest nodules were found under isolate 51 and the lowest number was found under isolates 21 and 47.

Isolate 9 showed the least number of nodules when fertility of soil increased to F3. At this level of fertility, isolates 3 and 21 showed the highest number of nodules. Furthermore, increase of soil fertility resulted in more variation among nodules number of the isolates. In this regard, number of nodules for each isolate at the highest fertility level was the least for isolate number 51. However, the number of nodules for isolate 21 and 47 were equal.

These results confirm the previous ones that the number of nodules may not reflect the percentage of BNF by isolates. These results agree with those of Bantilan and Johasen [10].

According to statistical analysis, the LSD for nodules under fertility levels was 1.1435; LSD for nodules of six isolates under fertility levels was 1.5127; LSD for nodules as affected by isolate-fertility interaction was 3.1167. The data showed that fertility levels are of significant effect on average nodules number of all isolates. Significant differences at $P \leq 0.01$ were observed among nodules numbers of the six isolates as affected by fertility level. Nodules number of each isolates under different fertility levels is significantly varied.

3.3 Effect of Soil Fertility Levels on Plant Dry Weight

Results in Fig. 3 showed that the least plant dry weight was under the first fertility level irrelevant of *Rhizobium* isolates. In general, the highest plant dry weight was under the second soil fertility level.

This may indicate that the nutrients added in this level represent the plant sufficiency level [13] without affecting the isolates efficiency of BNF. However, nitrogen fixing isolates may supply the plant with what is requested by plant.

The high plant dry weight, on the other hand, under F1, F2, F3 and F4 were observed under isolate 26, 9, 21 and 3, respectively. These results are very much comparable to that of BNF% as well.

Statistical analysis of the data showed that the LSD for dry weight under fertility levels was 0.4738; LSD for nodules of six isolates under fertility levels was 0.6268; LSD for nodules as affected by isolate fertility interaction was 1.2813. It showed that there is significant
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Fig. 3  Effect of soil fertility levels on plant dry weight (g/pot) under local isolates of *Bradyrhizobium*.

variation in plant dry weight under fertility F1, F3 and F4. However, there is no significant difference between the 3rd and 4th fertility level.

4. Conclusions

Under the same soil fertility level, isolates showed measurable differences in their ability to fix N. Isolates showed different level of BNF under various soil fertility levels evaluated in this soil. BNF of all isolates markedly reduced with the increase of soil fertility level. These results confirm that the number of nodules did not reflect the percentage of BNF by isolates. The results also suggested the need to determine the critical level of N in soils at which BNF is at the maximum. This will tremendously reduce the level of N fertilizer additions.

References


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