LETTER TO THE EDITOR

How to manage a scientific project in biological nitrogen fixation (BNF) and the topics that needed to be searched?

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Abstract
Planning to have a good scientific project generally or specifically in BNF is a main target for young scientists and PhD students worldwide. Therefore, it is hardly urgent to well manage the project, which defined the use of knowledge, skills and methods to achieve objectives of the project in the determined time without delay. Consequently, in this letter I will explain the main points that needed to be covered to manage a research project in BNF and which subjects of research are requested to be covered in the future.

Keywords Biological nitrogen fixation (BNF), Project management, Research points in BNF

Background
Symbiotic nitrogen fixation is a very important scientific branch that means the symbiosis life between microbial symbionts and their legume partners under a specific deficiency of available nitrogen in the soil (Linstrom and Mousavi 2020). In this symbiosis life, each partner introduces a benefit to another. For example, the legume plant supports the bacteria of the dominant genus *Rhizobium* with carbohydrate that is fixed through Calvin cycle (photosynthesis process); however, microbial symbionts of genus (*Rhizobium*, *Ensifer*, *Bradyrhizobium*, *Paraburkholderia*, etc.) (Shamseldin et al. 2017) support the plant with ammonium after fixing the nitrogen gas from the atmosphere. To start a scientific project in this interested field of research, it is obligatory to know how to manage it and the urgent research points.

Steps for project management
Different important questions are necessary to be answered to write and manage a project in this area of research such as outlined points in Fig. 1.

1. Which legume crop do you need to study? Choose the legume crop.

   This is differed from country to another depending on the importance of this crop to the human or animals in this country. Commonly edible crops like bean, pea, lentil, faba bean, soy bean, mung bean, chickpea and lupine are important plants in the developing countries to solve the gap between production rate and local consumption of foods in these countries. On the other side, forage legume such as clover is important for societies that used it for animal feeding like situation in Egypt (Rady et al. 2022), because clover occupies about 60% of the cultivated area in the winter season.

2. What are the problems do you intend to solve in this crop? Define the problems.

   Some legume crops have the endogenous *Rhizobium* strains in the rhizosphere; however, these strains are unable to form nodules or form less nodules due to
their less competitiveness associated with environmental stresses as high salinity level, elevated temperature, alkalinity and low temperature in the cold countries. In this case, it is urgently to focus on the selection of strains that are more adapted and competitive under these circumstances. On contrary to this, other soils are suffered from the absence of effective rhizobial strains at all in the soil (Daniele and Louise 2023).

3. How long of time do you need to implement your project? **Time table.**
Normally the less period of time to achieve a project in this area is two years which can be extended to three or four years according to the achievements that the group of research is planning to do. This is also correlated with the cultivation time of this crop and its rotation system. For better management of the time to achieve the project, we need to a—select the co-partners and their assistances very well, b—distribute the roles among co-partners appropriately, c—give a priority to a achieve the tasks, d—solve the appeared problems on timely, and e—respect strongly the time table and do not allow any delay.

4. How many partners do you need to help for implementing this project? **Number of partners.** It depends on the number of goals in the project and the contribution of each partner. Sometimes you need to contact foreign partners based on where do you plan to get the fund of the project.

5. How do you plan to obtain the fund and what about the size of the fund? **Financial of the project.** To obtain a fund for your project, you need to decide the type of fund. Is it internal or external outside from your country? The external fund will need to search for foreign scientific partners outside your home country. The size of fund depends on the size and type of equipment’s that you already have in your lab from infrastructure and the new machines that you miss in your lab, and the goals of the project.

6. How do you plan to get the microbial symbionts? **Collecting symbionts (Rhizobium).**
To get *Rhizobium* strains that will be used in your project, there are two ways to obtain microbial strains. The first is to collect nodules from fields that are cultivated with the selected legume crop of your project and isolate *Rhizobium* partners after surface sterilization of nodules as mentioned in detail by Erika et al. (2020). However, this way will lead to lose a part of time because the researchers should wait for the time of host cultivation. The other way is the host trapping, by collecting a part of soil you are interested in and using this soil as inoculums for the cultivated host in the lab; after 25 or maximum 30 days, you can collect nodules and start the isolation of microorganism after surface sterilization according to the same method mentioned above.

7. Who will be benefited from the project? **Identify the stakeholders.** It is urgent to figure out who the stakeholders are and who will use and benefit from the project’s output.

8. At the beginning of the research project (priority of the goals), it is necessary to put the goals of the

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**Fig. 1** The most important points that should be considered to manage a project in BNF
project which should be appropriate to the schedule of time. The priority should be given to isolate the *Rhizobium* strains (micro-symbionts) and their identification based on morphological and molecular tools. After that, the rest of the goals should be arranged according to their importance.

The urgent research points
Although researchers have done many publications and scientific achievements about nitrogen fixation in both symbiotic between legumes and their *Rhizobium* partners, and non-legumes, still there are some scientific points that needed to be covered or should have much attention in the future as listed and summarized in Fig. 2.

1. Why is the *Rhizobium* genome arranged in the way known for this (Shamseldin et al. 2021)? **Genome structure arrangement.**
2. Why is there a repeating of some specific genes in *Rhizobium* genome like *nif* genes (Wardell et al. 2022)? **Repeating of some important genes.**
3. Are there still important genes for nodulation and nitrogen fixation not discovered yet (Vlk et al. 2022)? **The function of not yet discovered genes.**
4. Why *Rhizobium* strains are appeared in different bacteroid stage and what is the mechanism responsible for this appearance (Coba de la Pena et al. 2018)? **Bacteroid shapes.**
5. Do the research efforts on *Rhizobium* and its partner improved legume production actually? **The success of rhizobial inoculation.**
6. What are the challenges that retard cereals from living in symbiosis life like legumes (Mus et al. 2016; Shamseldin 2022; Guo et al. 2023)? **Nitrogen fixation in cereals.** Of course, this will focus on the potentials of diazotrophs and endophytes to reduce the dependence of cereals on synthetic nitrogen fertilizers.
7. Do researchers success to move *nif* genes to cereals to make them fix nitrogen directly without nodulation (Shamseldin 2022)? **Expression of nif gene in non-legumes.**
8. How many legume species are studied and their *Rhizobium* partner identified (Shamseldin et al. 2017)? **Multiple of legumes not searched.**

Conclusions
To guarantee the success of this project, it is necessary to determine the requirements, objectives, persons and their skills, correct distribution of the roles among researchers who will be cooperated in this project, avoid the conflict of interest between the research team, evaluate the degree of performance of all persons periodically, and the most important thing is to start your work where the other scientific groups stopped; do not start from the beginning or repeating the effort done by others, meaning that it is important to study the new and relevant points which are mentioned above.

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**Fig. 2** Research subjects of BNF which are requested to study in the future
Abbreviations
BNF  Biological nitrogen fixation
PhD  Philosophy of Doctor
nif  Nitrogenase induced in nitrogen fixation

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