# Application of plant growth promoting rhizobacteria (PGPR) as biological fertilizers for improving the growth and yield of saffron (*Crocus sativus* L.)


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## Introduction

Plant growth promoting rhizobacteria (PGPR) represent today a new dimension for sustainable agriculture and environmental development, because they can enhance plant growth by a panel of different direct or indirect mechanisms [1][2][3].The application of the PGPR as biofertilizers offers a way to replace, at least partially, the use of chemical fertilizers and pesticides seeing their fatal effects on the environment and the human health. In the frame of this global approach, we have studied the functional and genetic diversity of the rhizobacteria colonizing the rhizosphere of saffron (*Crocus sativus* L.) (Figure 1) and their possible utilization as biofertilizers for saffron, one of the rarest and most expensive medicinal plant in the world, that is cultivated in different countries of Asia and the Mediterranean region [4].

**Figure 1:** Rhizosphere and corms of saffron

**The AIMS of the present research were:**

- Isolation of the rhizobacterial strains from the rhizosphere of saffron and their characterization for beneficial biological activities.
- The conception of the best bacterial inocula for saffron with the strains showing the higher levels of biological activities.
- Testing these biofertilizers in field inoculation trials for their capacity to improve saffron growth, its yield and quality.

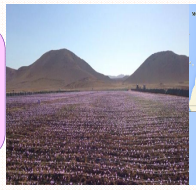

**Figure 2:** Farm 1.2.3. SAFRAN, Taliouine

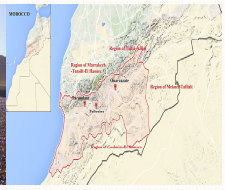

**Figure 3:** Location of production of the saffron in Morocco (Souss-Massa region)

## Material and Methods

**1.Collecting and analysis of saffron rhizospheric soil**: Samples of rhizospheric soil were collected in the farm "1.2.3. SAFRAN" (Figure2) situated in Taliouine (Souss-Massa region, Morocco) (Figure3) which is the major Moroccan saffron production zone. The soils were analyzed in the "Laboratoire des moyens analytiques (LAMA) of the Institute of research for development" (IRD) in Dakar (Senegal).

**2. Isolation and evaluation of the biological activities of the saffron isolates:** Isolation of the rhizobacteria was performed on three different media: Pikovskaya's medium (PVK), MODI medium and YEM-try medium. The isolates were tested for PGP activities: auxin synthesis, siderophore production, inorganic phosphate solubilization and atmospheric nitrogen fixation [5, 6, 7, 8].

**3. Formulation of microbial biofertilizers with selected PGPR strains:** The best three isolates, with highest levels of biological activities (S11P1,S12S4 and S11A1a) were used for the production of saffron's biofertilizers and were tested in field trials in the farm "1.2.3. SAFRAN".

4. **Statistical analysis:** The analysis of variance (ANOVA) of the data obtained was performed using the software XLSTAT.

## Results and discussion

- Soil analysis indicated that saffron is cultivated in a very poor alkaline sandy clay loam soil that needs to be supplemented with organic matter and mineral fertilizers for improving its fertility.

**Table 1**: Physico-chemical characteristics of the saffron soil

| | | | |
|---|---|---|---|
| **GRANULOMETRY** | | Total nitrogen (%) | 0,04 |
| **Clays (%)** | 21,80 | Total carbon (%) | 0,89 |
| **Fine silt (%)** | 11,90 | C / N | 22 |
| **Coarse silt (%)** | 11,20 | Available P (mg/kg) | 18,0 |
| **Fine sands (%)** | 43,20 | p $H_2O$ | 8,3 |
| **Coarse-grained (%)** | 11,40 | H KCl | 7,6 |

- Bacterial isolation permitted us to constitute a collection of 90 rhizobacterial isolates (35 strains on PVK medium (39%), 22 strains on MODI medium (24%) and 33 strains on YEM-try medium (37%)).

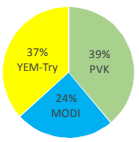


**Figure 3**: Distribution of the isolated bacteria in selective media

- The best three isolates, showing higher levels of biological activities, were selected and used for the formulation of saffron biofertilizers (**S11P1**, **S12S4** and **S11A1a**). The field experimental trial used was a randomized complete block (Figure 4), carried out in an area of approximately 1575 $m^2$ situated in the farm 1.2.3. SAFRAN (Taliouine, Morocco). **Two years** after the plantation of pre-inoculated bulbs of saffron with PGPRs, we have measured beneficial effects of the bacterial inoculation on some parameters.

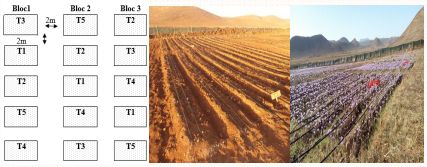


**Figure 4:** Field inoculation trial in the farm 1.2.3 SAFRAN in Taliouine

Experimental design: randomized complete block with five inoculation treatments; **T1**: Control, **T2**: inoculation with the strainS11P1, **T3**: inoculation with the strain S12S4, **T4**: inoculation with the strain S11A1a and **T5**: inoculation with a bacterial mixture (S11P1/S11S4/S11A1a).

The statistical analysis showed that inoculation with the rhizobacterial strain S11P1 had an observable and significant effect compared with the control, especially on the mother corms number (Figure 5), a positive effect on the length of leaves (Figure 6), on the concentrations of chlorophyll a, chlorophyll b and total chlorophyll (Figure 7) and on the other growth parameters. One of the most important traits of the rhizobacteria S11P1 is its ability to release high amounts of soluble phosphate from rock phosphate (166.04mg/l ±14.4mg/l of medium). The second PGPR strain tested (S12S4) had a positive and significant effect on the biomass of saffron plants. It increases the fresh and dry weights of leaves, the fresh and dry weights of mother and daughter corms. This strain had also a positive effect on chlorophyll concentration and other growth parameters of saffron plants (Table 2 and 3). The last rhizobacterial strain S11A1a, which showed a very interesting *in vitro* production of auxin (124.36 µg/ml), had also positive effects on saffron in the field. It increases the number of leaves and their fresh weight, the number of daughter corms (Figure 8) and their fresh and dry weights, and the chlorophyll content (a, b and total).

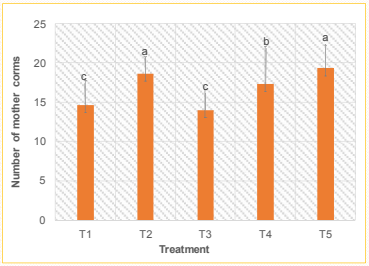


**Figure 5:** Effect of the inoculation with PGPR on the number of mother corms of *Crocus sativus* L.

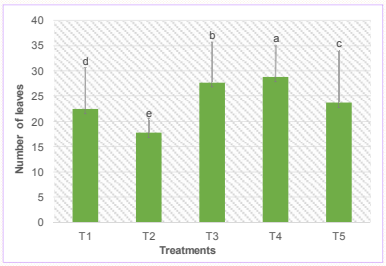


**Figure 6:** Influence of rhizobacterial strains on the number of leaves of *Crocus sativus* L.

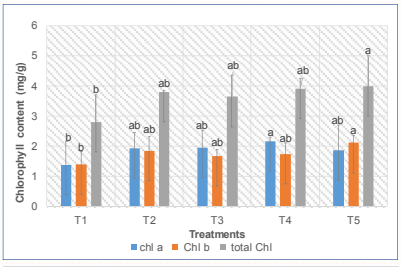


**Figure 7:** Effect of the inoculation with PGPR on the chlorophyll content in leaves of *Crocus sativus* L.

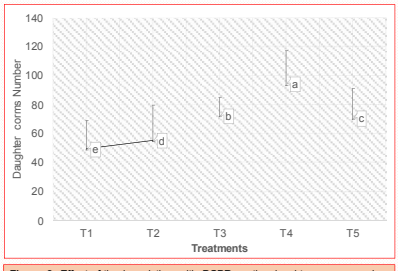


**Figure 8:** Effect of the inoculation with PGPR on the daughter corms number

The primary goal of testing a bacterial mixture was to see if it is possible of gathering the properties of these three PGP rhizobacteria (S11P1, S12S4 and S11A1a) in only one inoculum (treatment 5). The results obtained after the inoculation of the saffron with the mixture, show an effect on the photosynthetic efficiency by increasing the concentration of chl a, chl b and total chl, and on the increase of the length and biomass of leaves.

**Table 2:** Effect of the inoculation by the PGPR on the biomass of aerial parts of *Crocus sativus* L.

| Treatments | Wet weight of the aerial parts (g) | Dry weight of the aerial parts (g) |
|---|---|---|
| **T1** | 2.31±0.5ab | 0.68±0.1bc |
| **T2** | 1.71±0.2b | 0.52±0.05d |
| **T3** | 2.83±1.5a | 0.77±0.3a |
| **T4** | 2.57±0.2ab | 0.7±0.04b |
| **T5** | 2.19±1.3ab | 0.62±0.3c |

**Table 3:** Effect of the inoculation by the PGPR on daughter corms biomass

| Treatments | Daughter corms fresh weight (g) | Daughter corms dry weight (g) |
|---|---|---|
| **T1** | 9.83±4.3c | 3.54±1.56bc |
| **T2** | 6.74±1.32d | 2.51±0.48c |
| **T3** | 12.6±6.43a | 4.74±2.33a |
| **T4** | 11.35±2.72b | 4.28±0.82ab |
| **T5** | 8.99±5.93c | 3.33±2.23bc |

## Conclusion

In the present study, some rhizobacterial strains were used for the formulation of biofertilizers that were inoculated to saffron bulbs and tested in field trials during 2 growing seasons (2013 - 2015). The results obtained show a beneficial impact of these strains on the growth and quality of saffron. We can postulate that the rhizobacteria tested were very active under the adverse conditions prevailing in the region of Talouine, probably by affecting plant nutrition and/or growth stimulation through the expression in the field conditions of their plant growth promoting activities revealed *in vitro*. Our results suggest that selected higher performing rhizobacteria could be used successfully as biofertilizers for *Crocus sativus* L. cultivated under traditional organic farming systems.


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