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Improving Quality of *Crocus Sativus* Through the Use of *Bacillus Subtilis*

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Abstract- In this study the ability of *B.subtilis* to improve the quality of *C.sativus* cultivation was evaluated. Various scientific studies demonstrate the medicinal properties of its constituents, making it one of the most expensive agricultural products in the world. The presence of microorganisms in the soil promotes the growth of plants, thanks to this capacity, today there are several microbial products marketed that can increase the health and growth of plants. Several scientific researches have demonstrated the beneficial effects of rhizosphere bacteria on roots and overall plant growth. In this study the ability of *B.subtilis* to improve the quality of *C.sativus* cultivation was evaluated. The two experimental groups in cultivation were: i) group without beneficial micro-organisms, irrigated with water and substrate previously fertilized; ii) group with beneficial micro-organism *B.subtilis,* irrigated with water and substrate previously fertilized. Growth-promoting bacteria and in particular *Bacillus subtilis* have important effects on plant morphology even under stressful conditions. In this experiment it is noted above all that the substrates enriched with *B.subtilis* for the cultivation of *Crocus sativus* led to a significant increase in all the agronomic parameters analyzed (length and leaves number, vegetative and root biomass, bulb weight, flowers number and stigmas length). This confirms how the use of *B.subtilis* can improve the solubility and availability of nutrients that improve plant growth accordingly.

Keywords- Microorganisms; Sustainable applications; Crocus sativus; Rhizosphere, PGPR

I. INTRODUCTION

The genus Crocus (Family: Iridacae) is composed of about 80 different species [1], grown worldwide mainly for medicinal use and in the paper and tissue colouring industry.
C. sativus L. is cultivated all over the world, mainly in countries such as Spain, India, Turkey, Greece, Austria, Belgium, France, Germany, Holland, Italy, Japan, Norway, Russia, Switzerland, Turkey, Persia and the Republic of China [2,3]. To grow at its best, the crop needs crumbly, loose, low-density, well irrigated and well drained, medium fertility calcareous clay soils. The work of genetic improvement and cultivation techniques has led to more flowers and an increase in the production of stigmas, which are so commercially attractive [4]. The planting methods and corm size have also had a positive influence on increasing *C.sativus* production and selecting high quality germplasm, as well as the size of the mother corm, the cultivation method (greenhouse compared to field), fertilizers and water availability. The use of cold during cultivation has led to a significant increase in the number of flowers and at the same time a lower production quality of stigmas. Planting takes place from spring to autumn and they remain in the soil for at least ten years before being replaced [5]. Flowering lasts only a few weeks and harvesting must take place every day to obtain quality stigmas. Various scientific studies demonstrate the medicinal properties of its constituents, making it one of the most expensive agricultural products in the world [6].

The presence of microorganisms in the soil promotes the growth of plants, thanks to this capacity, today there are several microbial products marketed that can increase the health and growth of plants [7]. Several scientific researches have demonstrated the beneficial effects of rhizosphere bacteria on roots and overall plant growth [8,9,10,11]. In general these beneficial bacteria are defined as plant growth promoting rhizobacteria (PGPR). The beneficial effect can occur through both direct and indirect mechanisms [12]. Direct methods involve the production of metabolites and compounds that stimulate vegetative and root growth of plants and increase tolerance to biotic and abiotic stress. Plant growth is also influenced by the induction of systemic resistance and hormonal stimulation mediated by microorganisms [13].

II. RELATED WORK

Microorganisms use different techniques to colonize the rhizosphere, in particular *B.subtilis* forms a thin biofilm on the roots that can facilitate long-term colonization, in particular by localizing and colonizing young roots [14]. *Bacillus subtilis* can stimulate plant growth by producing substances such as indolic acetic acid, solubilize phosphates, ammonium and producing siderophores, for this reason today it is widely used in the cultivation and defense of plants, especially those whose aesthetic and organoleptic quality is more influenced by biotic and abiotic factors [15].

In order to improve the quality and quantity of agricultural production without adversely affecting the biodiversity of the agro-system, there is an increasing need to use microbial inoculants that promote plant growth, disease control agents and soil health enhancers. PGPRs also play an important role in improving plant health, resistance to environmental stress, and soil bioremediation. In this study the ability of *B.subtilis* to improve the quality of *C.sativus* cultivation was evaluated.

Figure 1 – detail of the radical growth and flower of *Crocus sativus*

III. METHODOLOGY

The experiments, started in July 2019, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Crocus sativus* bulbs. The bulbs were placed in ø 12 cm pots; 30 bulbs per thesis, divided into 3 replicas of 10 bulbs each. All bulbs were fertilized with a controlled release fertilizer (3 kg m-3 Osmocote Pro®, 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The two experimental groups in cultivation were:

- Group without beneficial micro-organisms (CTRL) (peat 70% + pumice 30%), irrigated with water and substrate previously fertilized;
- Group with beneficial micro-organism *B.subtilis* (BSUB) (peat 70% + pumice 30%), irrigated with water and substrate previously fertilized, (30mg SuperSmart Bacillus per litre of soil, $3x10^9$ cfu).

The plants were watered 2 times a week and grown for 7 months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the fraction of leaching. On January 15, 2020, the leaves lenght, leaves number, bulbs weight, the vegetative and root weight, flowers number and stigmas lenght were recorded.

Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

IV. RESULTS AND DISCUSSION

All *Crocus sativus* plants treated with *Bacillus subtilis* showed a significant increase in the agronomic parameters analysed compared to the untreated control (CTRL). The results show that the use of a rhizobacterium such as *B.subtilis* can improve the quality of treated plants by increasing water and nutrient uptake.

In *Crocus sativus*, (Table 1) the leaf length was 36.46 cm in (BSUB), significantly better than 23.98 cm in the untreated control (CTRL).

Also the leaf number, 13.00 (BSUB) was significantly better than the control (CTRL) with 11.00 leaves.

There was a significant increase in vegetative biomass 4.70 g (BSUB), compared to the untreated control 3.62 g (CTRL). Also the treatment (BSUB) for the root biomass was significantly better with 3.46 g, compared to 2.24 g of the control (Figure 2).
Treatment with Bacillus subtilis resulted in a significant increase in bulb weight of 8.04 g compared to 6.78 g of the untreated control (Figure 3). It also resulted in a significant increase in the number of flowers 2.20 compared to 1.21 in the control (Figure 4).
It also showed, very interestingly, a significant increase in stigma length in the thesis treated with (BSUB), 6.10 cm compared to 5.22 cm in the untreated control.

Table 1 - evaluation of Bacillus subtilis on agronomic characters on plants of Crocus sativus

Groups	**Leaves lenght (cm)**	**Leaves number (n°)**	**Vegetative weight (g)**	**Roots weight (g)**	**Bulbs weight (g)**	**Flowers number (n°)**	**Stigmas lenght (cm)**
CTRL	31,07 ^{b}	11,00 ^{b}	3,62 ^{b}	2,24 ^{b}	6,78 ^{b}	1,21 ^{b}	5,22 ^{b}
BSUB	36,46 ^{a}	13,00 ^{a}	4,70 ^{a}	3,46 ^{a}	8,04 ^{a}	2,20 ^{a}	6,10 ^{a}
ANOVA	***	**	***	***	***	**	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$)

Figure 2 - Effect of Bacillus subtilis on vegetative and roots biomass of Crocus sativus. Legend: (CTRL) control; (BSUB) Bacillus subtilis

Figure 3 – Effect of *Bacillus subtilis* on bulbs weight of *Crocus sativus*
Legend: (CTRL) control; (BSUB) *Bacillus subtilis*

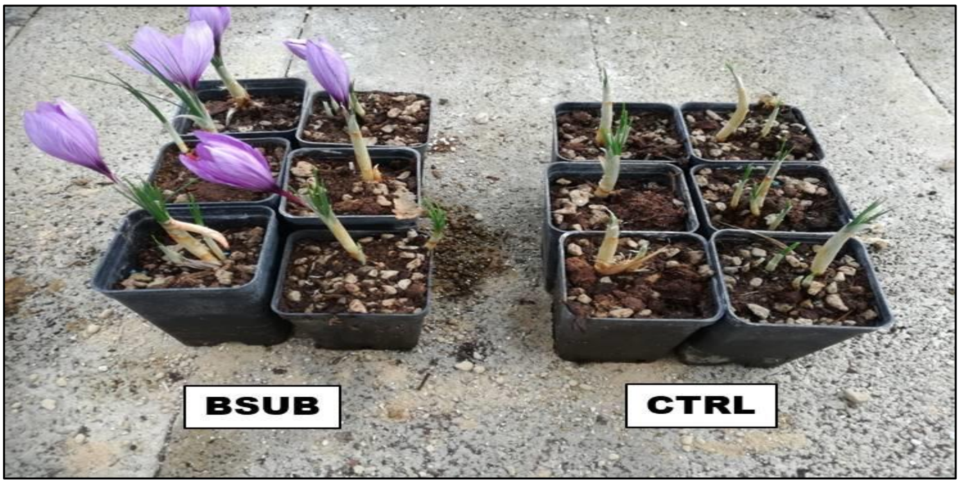

Figure 4 – Effect of *Bacillus subtilis* on flowering plants of *Crocus sativus*
Legend: (CTRL) control; (BSUB) *Bacillus subtilis*

The place in the soil where nutrient uptake takes place and important physiological, chemical and biological activities take place is the rhizosphere, where the roots grow [13].
Plant roots receive 30 to 60% of the carbon produced by photosynthesis. Of this, estimated percentages between 40 and 90% penetrate the soil in the form of various compounds such as alcohols, ethylene, sugars, amino acids, organic acids, vitamins, nucleotides, polysaccharides and enzymes [16]. Microorganisms in the rhizosphere increase their number when substrates synthesized by plants and released by roots become bioavailable; the composition and function of the microbial community also changes. In addition, microorganisms in the rhizosphere act as stable sources of nutrients for other organisms and thus play a critical role in the synthesis and degradation of organic matter, generating a microbial cycle in the soil. A wide range of microbial species in the rhizosphere can promote plant development, due to their ability to communicate with the plant using complex chemical signals [8].

The Bacillus species has the ability to form highly resistant spores and produce metabolites that stimulate plant growth and prevent the development of pathogens. The inoculation of microorganisms in the rhizosphere represents a technique to increase tolerance to abiotic stress, in particular those caused by climate change [17]. Bacillus can play a significant role in tolerance to this type of stress and has the ability to secrete exopolysaccharides and siderophores capable of inhibiting the movement of toxic ions by sending ionic balance, promoting water movement in plant tissues and inhibiting the growth of pathogenic microorganisms [18].

Growth-promoting bacteria and in particular *Bacillus subtilis* have important effects on plant morphology even under stressful conditions. In this experiment it is noted above all that the substrates enriched with *B.subtilis* for the cultivation of *Crocus sativus* led to a significant increase in all the agronomic parameters analyzed (length and number of leaves, vegetative and root biomass, bulb weight, number of flowers and length of stigmas). This confirms how the use of *B.subtilis* can improve the solubility and availability of nutrients that improve plant growth accordingly [19].
Bacillus spp. are endospore producing bacteria that can help bacteria survive in harsh environmental conditions. There are several beneficial effects that can be allowed to the plant following colonization by *Bacillus subtilis*: increased seed germination rate, improved aesthetic and productive quality of plants, physiological and hormonal stimulation of plants, increased resistance to biotic and abiotic stress.

V. CONCLUSION

The test has shown that the use of *Bacillus subtilis* can significantly improve the agronomic quality of *Crocus sativus*. Rhizobacteria and in particular *B.subtilis*, once inoculated into the growing medium, improve water and fertilizer uptake by the roots and play a key role in influencing the physiological and hormonal aspects of plants.

The most important applications of PGPR and *Bacillus subtilis* to promote plant growth are in agriculture, horticulture, ornamental plants, succulents and cacti and to restore soil fertility. The effects most commonly found concern increases in seed germination rate, vegetative and root development, leaf area and chlorophyll content, nitrogen and protein content, tolerance to water, salt and abiotic stress in general, and improvement in the quality of field crops. These are very interesting aspects, especially when plant species are cultivated in environments other than native ones, but which have a great commercial interest.

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