Scientia Horticulturae 319 (2023) 112155

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Beneficial microorganisms: a sustainable horticultural solution to improve the quality of saffron in hydroponics

Stefania Stelluti [a], Matteo Caser [a], Sonia Demasi [a], Esteban Rodriguez Herrero [b], Irene García-González [b], Erica Lumini [c], Valeria Bianciotto [c], Valentina Scariot [a,*]

[a] Department of Agricultural, Forest and Food Sciences, University of Torino. Largo Paolo Braccini 2, Grugliasco, TO 10095, Italy
[b] Ceres Biotics Tech S.L., Mar Tirreno 8, Naves C27-C29, San Fernando de Henares 28830, Madrid, Spain
[c] Institute for Sustainable Plant Protection, National Research Council, c/o Department of Life Science and System Biology, University of Turin. Viale Mattioli 25, Torino 10125, Italy

ARTICLE INFO

Keywords:
Crocus sativus L.
AMF
PGPR
Safranal
Photosynthetic system
Corm starch

ABSTRACT

Saffron (*Crocus sativus* L.) is the most expensive spice in the world. Its organoleptic properties are mainly dictated by the apocarotenoids crocins (dyeing capacity), picrocrocin (pleasant taste), and safranal (scent). Cultivation of saffron in controlled conditions with beneficial microorganisms may increase its profitability. In this study saffron was grown in hydroponics under a greenhouse with the PGPR *Bacillus megaterium* CB97032 and *Paenibacillus durus* CB1806 (Pgpr treatment), and the AMF *Rhizophagus intraradices* (Myc treatment), alone or mixed (Mix treatment). The influence exerted during all crop phases on flowering trend, flower and spice yield, secondary metabolites, ecophysiological traits, and corm production was investigated, with the hypothesis that microbial synergy would have more positively affected these parameters. All the bioinoculants did not positively influence flower and spice yield, but enhanced the content of safranal (up to +96% in all the treated plants). When mixed, the PGPR and AMF improved the total phenolic content (+19%) of the saffron spice. Even if no differences emerged from the ecophysiological analysis, the corm yield was improved for the inoculated plants. The single-type bioinoculants allowed to obtain a higher number of replacement corms (up to +13% for the Myc plants) without reducing their weight, but lowering their size. When mixed, the size of the corms was restored. Together, the PGPR and AMF also increased the corm weight (+24%) of the largest corm fraction (> 1.5 cm). Thus, the bioinoculants may have stimulated the secondary metabolism of the plants by improving quality traits, rather than having acted as biofertilizers by increasing yield, at least during flowering. Overall, AMF and PGPR were proved to be a sustainable horticultural solution in hydroponics to improve the quality of saffron, especially when applied in mixed formulations.

1. Introduction

Saffron (*Crocus sativus* L.) is a subhysteranthous geophyte of the Iridaceae family. This sterile herbaceous plant multiplies through underground corms, which storage nutrient reserves and usually bear 1–3 flowers (; Kumar et al., 2008; Stelluti et al., 2021). In Mediterranean climates, saffron blooms for two to three weeks between early and late autumn. After flowering saffron enters a vegetative stage during which the leaves are photosynthetically active, providing nutrients for the formation of new corms (Renau-Morata et al., 2012).

Flowering is mainly regulated by thermoperiodicity and corm size (Gresta et al., 2008). Optimal flower formation can be achieved incubating corms at warm temperature (23 - 27 °C) for more that 50 days and less than 150 days for flower induction and at mid-low temperature (15 - 17 °C) for flower emergence (Molina et al., 2005a). Flower yield increases with larger corms; commercially, corms of 2.5 - 3.5 cm in diameter and 10 - 20 g in weight are usually selected (Caser et al., 2019). The spice of saffron is obtained by dehydrating the red stigmas of its ephemeral flowers. The daily manual harvest of flowers and separation of stigmas causes it to have the highest cost among spices (Caser et al., 2020). Saffron has earned the nickname "red gold" and unsurprisingly, its price ($40 - 50 g^{-1} Khan et al., 2020) approximates that of gold ($55 g^{-1}, goldprice.org, November 2022). The spice has been used to flavor and color food for centuries. Its pleasant bitter taste, inebriating scent,

* Corresponding author.
E-mail address: valentina.scariot@unito.it (V. Scariot).

https://doi.org/10.1016/j.scienta.2023.112155
Received 10 January 2023; Received in revised form 14 April 2023; Accepted 13 May 2023
Available online 23 May 2023

and dyeing capacity are mainly dictated by three apocarotenoids derived from zeaxanthin: picrocrocin, a glucoside of safranal (taste); safranal, a volatile monoterpene aldehyde (scent); and crocetin glycosides named crocins (dyeing capacity). *Trans*-crocetin di-(β-D-gentiobiosyl) ester and *trans*-crocetin di-(β-D-glucosyl)-(β-D-gentiobiosyl) ester are among the main crocins found in saffron (Carmona et al., 2006; Chen et al., 2020; García-Rodríguez et al., 2017; Tarantilis et al., 1994). These compounds are used to classify the spice into categories of quality (I, II, and III) according to ISO 3632 (2011) (Caser et al., 2018, 2019, 2020).

Saffron is mainly cultivated in the Middle East and Mediterranean regions. About 90% of total world production (418 t y^{-1} in 2018) comes from Iran and the remainder mainly from India, Afghanistan, Greece, Morocco, Spain, and Italy (Cardone et al., 2020). In the last century the saffron produced in Spain, Italy, and Greece has seriously decreased, mostly because the technology has not progressed for this plant's cultivation and the manual labor cost has increased (Cardone et al., 2020; Molina et al., 2005a). To make saffron more profitable in European areas, shifting its cultivation to controlled environments has been proposed (Askari-Khorasgani and Pessarakli, 2019; Avarseji et al., 2013; Caser et al., 2019; Molina et al., 2005b; Salas et al., 2020). The flowering period could be extended and crop management facilitated and improved with suitable nutrient solutions and by growing plants without pests or pathogens (Caser et al., 2019; Molina et al., 2005b; Salas et al., 2020). Further, controlled conditions may counteract the negative impact of global soil degradation and climate change (i.e., salinity and higher temperatures) and increase land-use efficiency (Askari-Khorasgani and Pessarakli, 2019).

The search for sustainable practices that can provide yields comparable to those of high-intensity agriculture continues to cope with the effects of climate change and reduce environmental costs (Rouphael and Colla, 2020). After the green-revolution, there is a need for the so-called microbial revolution, based on the utilization/manipulation of plant microbiota as a sustainable tool to enhance plant productivity (Backer et al., 2018; De Pascale et al., 2017; Genre et al., 2020). The use of beneficial microbes in agriculture begins in the early 20th century with the rhizobia (plant growth promoting rhizobacteria, PGPR) and several species of the Fabaceae family (Backer et al., 2018). PGPR comprise various genera, such as *Bacillus, Paenibacillus, Azospirillum*, and *Azotobacter*, with relevant properties, primarily N-fixation and P-solubilization, but also siderophore and phytohormone production and biological control. In the last decade, many formulations have been applied to different crops (Backer et al., 2018; Lobo et al., 2019). However, only N-fixing bacteria of the genera *Rhizobium, Azotobacter*, and *Azospirillum* are currently considered "plant biostimulants" (Regulation EU 2019/1009). This category includes also the arbuscular mycorrhizal fungi (AMF), subphylum Glomeromycotina, which establish mutualistic symbiosis with most land plants. The fungus receives photosynthesis-derived carbon and, in exchange, increases the uptake of water and mineral nutrients (such as P and N) by plants and enhances their tolerance to biotic and abiotic stresses, positively affecting plant productivity (Genre et al., 2020; Lanfranco et al., 2018). AMF are largely used in horticulture, particularly species of the genera Rhizophagus and Funneliformis, as they are generalist, widely distributed, and can be extensively propagated (Berruti et al., 2016; Giovannini et al., 2020). The upgraded plant nutritional status induced by beneficial microorganisms has been associated with increased content of secondary metabolites of interest and/or plant production in several crops, such as *Zea mays* L., *Solanum lycopersicum* L., *Capsicum annuum* L., *Ocimum basilicum* L., *Mentha* spp., *Echinacea purpurea* (L.) Moench., *Artemisia annua* L., *Stevia rebaudiana* (Bertoni) Bertoni, *Allium sativum* L., *Hypericum perforatum* L., and also in *Crocus sativus* L. (Backer et al., 2018; Bianciotto et al., 2018; Kour et al., 2018; Kumar et al., 2021; Pandey et al., 2018; Rouphael et al., 2015). Nutrient availability, especially of N and P, mainly affects the growth of saffron corms during the vegetative phase (Koocheki and Seyyedi, 2015). AMF and P-solubilizing bacteria can work synergistically improving P availability and P uptake by plants (Etesami et al., 2021; Giovannini et al., 2020). Particularly, in soilless cultivation system where low P nutrient solutions are usually used to avoid inhibition of AM symbiosis , e.g., in experiments with *Solanum lycopersicum*, 300 μM in Volpe et al. (2018) or 3.2 μM in Chialva et al. (2020), Mannino et al. (2020), and Zouari et al. (2014). The growth promoting effects may be more beneficial when diazotrophic bacteria are added to the microbial mix, which could also further reduce the use of chemicals (Lobo et al., 2019). Moreover, *Paenibacillus* spp. and *Bacillus* spp. can stimulate the growth of AMF and potentially promote the establishment of symbiosis (Rouphael et al., 2015).

The rhizomicrobiota composition is strictly controlled by plants, which select the most beneficial microbes by releasing root exudates and signal compounds (Backer et al., 2018; Genre et al., 2020; Victorino et al., 2021). Out of various PGPR recently isolated from saffron rhizosphere, *Bacillus megaterium* and *Paenibacillus* sp. presented multiple growth promoting traits and positively affected the plant production (Jami Al-Ahmadi et al., 2017; Kour et al., 2018). Chamkhi et al. (2018) characterized the AMF associated with saffron, most frequently finding the genus *Rhizophagus* (Walker et al., 2021) within the roots. So far, the responses of saffron to PGPR or AMF have been still poorly investigated in hydroponics, focusing mainly on quantitative productive traits (Ambardar and Vakhlu, 2013; Caser et al., 2019; Kour et al., 2018; Magotra et al., 2021) and only a few studies (Caser et al., 2019; Sharaf-Eldin et al., 2008) on secondary metabolites of the spice. As regards the productive traits, the spice yield was not influenced by AMF (*Rizophagus intraradices* and *Funneliformis mosseae*) (Caser et al., 2019), but it was increased by PGPR inoculants, e.g. *Pseudomonas* spp. and *Bacillus* spp. (Díez-Méndez and Rivas, 2017; Magotra et al., 2021; Sharaf-Eldin et al., 2008). The corm yield (size, number of replacement corms, and/or weight) was improved by both AMF and PGPR formulations (Ambardar and Vakhlu, 2013). Regarding the phytochemicals in saffron, the content of crocins was reduced by *Bacillus subtilis* FZB24® (Sharaf-Eldin et al., 2008), which conversely increased the content of picrocrocin, crocetin, and safranal. To our knowledge, no studies have investigated the effects of beneficial microorganisms on saffron photosynthesis, which provides nutrients for corm growth. The aim of this study was to deepen the knowledge on the influence exerted during all crop phases by different beneficial microorganisms, investigating saffron yield and secondary metabolites, ecophysiological traits, and corm production in hydroponics. Saffron was grown in a greenhouse using PGPR with different principal capacity of plant growth promoting, i.e., *Bacillus megaterium* CB97032 (P-solubilizer) and *Paenibacillus durus* CB1806 (N-fixing), not yet tested on saffron, and the AMF *Rhizophagus intraradices*, alone or mixed. The bipartite (i.e., a plant interacting with a single type of microbe, saffron-PGPR/AMF) and tripartite (saffron-PGPR-AMF) interactions were investigated. The hypothesis was that the microbial synergy in the mixed formulation would affect saffron production, quality-related compounds, and the photosynthesis process more positively than single-type inoculum.

2. Materials and method

2.1. Plant materials and cultivation conditions

The experiment took place in an unheated greenhouse at the Department of Agricultural, Forest, and Food Sciences (DISAFA) of the University of Turin (Italy, 45°06′23.21″N Lat, 7°57′82.83″E Long; 300 m a.s.l.). Saffron grows well in drained soils with pH 6.8- 7.8, and electrical conductivity (EC) <2 dS m^{-1} (Salas et al., 2020; Gresta et al., 2008). Large-sized corms (≥19 g) were sowed on 31 August 2020 in pots (4 L, 14 × 14 cm side, and 17 cm height; one corm per pot) filled with sterile expanded perlite (compacted density of 120 ± 25 kg m^{-3}; granulometry of 2 - 6 mm; 1.5 L per pot; Centro Evergreen Turco s.a.s., Moncalieri, Turin, Italy). Out-of-trial pots allowed to visualize phenological changes of the corms. Irrigation water (pH 7.4, EC 505 μS cm^{-1}; SMAT,

Grugliasco, Turin) was added weekly from corm planting to root emergence (200 mL per pot). Subsequently, fertigation with a modified Long-Ashton solution (Hewitt, 1952) as in Chitarra et al. (2016) (Table 1) was carried out every 2 weeks until leaf senescence in spring (200 mL per pot). The solution had a low P concentration (300 μM) to avoid inhibition of AM symbiosis (Chitarra et al., 2016). The pH was adjusted by adding H_2SO_4 0.1 N (pH 7, EC 979 μS cm^{-1} at 22 °C).

A randomised block design was used with three replicates (consisting of three blocks) per treatment. Corms were inoculated with AMF (Myc treatment), PGPR (Pgpr treatment), and a mixture of AMF and PGPR (Mix treatment); not-inoculated corms were the controls (Ctr). Each block was composed of 12 pots per each treatment and 6 pots for the controls, for a total of 126 pots on a greenhouse bench. The AMF inoculum (MycAgro Lab, Breteniére, FR) consisted of *Rhizophagus intraradices* spores and a substrate of calcined clay, vermiculite, and zeolite and ~10 g of inoculum was put under each corm. The formulations of the two PGPR species (Ceres Biotics Tech S.L., Madrid, Spain) *Bacillus megaterium* and *Paenibacillus durus* were mixed in the nutrient solution and applied three times during fertigation: at root emergence around the end of September (73.6 mg L^{-1}); two weeks afterwards (booster dose of 7.36 g L^{-1}); after two months from the first application (73.6 mg L^{-1}).

The air temperature and relative humidity in the greenhouse were daily monitored by a datalogger (Fig. 1). The daily mean temperatures during flowering are showed in Fig. 2. During flowering, the daily mean values of temperature and relative humidity (RH%) were 17 ± 3 °C and 65 ± 10%. During the two main flowering peaks, the daily mean values were 19 ± 3 °C and 67 ± 10% (first peak), and 18 ± 3 °C and 63 ± 11% (second peak).

Growing degree days (GDD) were calculated as follows:

$$GDD = \Sigma[(T_{max} + T_{min})/2 - T_{base}]$$

Where T_{max} and T_{min} are the maximum and minimum daily air temperature, respectively, and T_{base} is the base temperature (McMaster and Wilhelm, 1997). GDD were referred to flowering and T_{base} was identified at 10 °C.

2.2. Evaluation of AM colonization, bacterial presence, and PGP activities

During flowering, the roots of two plants per replicate were harvested, rid of topsoil, and cleaned. For each sample, part of the roots was used for biomass measurement and the remainder was stained to evaluate AM colonization. Briefly, saffron roots were stained with 0.1% (w/v) cotton blue in 90% lactic acid overnight and de-stained two times, with water (2 h) and 90% lactic acid diluted in deionized water 1:1 (v/v) (2 h). The roots were then left in 90% lactic acid. The protocol was performed twice to ensure a better staining. The roots were cut into fragments of ~1 cm and placed on microscope slides (20 fragments per slide) for further analysis under a light microscope (Trouvelot et al., 1986). For Myc and Mix plants, three slides per biological replicate were observed for a total of ~180 cm of root per treatment. For both Ctr and Pgpr plants, the absence of AMF was checked in one slide per biological replicate for a total of ~60 cm of root.

Two weeks after the end of flowering, the presence of the PGPR species and main PGP activities (i.e., fixed N_2 potential, P and K solubilization, and siderophore production) were analysed on a minimum of three rhizosphere samples of Ctr, Pgpr, and Mix plants. The bacterial concentration was observed in both general (TSA) and free-nitrogen (A6) media (Qaisrani et al., 2019).

Table 1
Composition of the modified Long-Ashton nutrient solution.

Elements	Concentration (mM)
$MgSO_4 \cdot 7H_2O$	0.75
$NaNO_3$	1
K_2SO_4	1
$CaCl_2 \cdot 2H_2O$	2
Na_2HPO_4	0.3
FeNa-EDTA	0.025
$MnSO_4 \cdot 12H_2O$	0.005
$CuSO_4 \cdot 5H_2O$	0.00025
$ZnSO_4 \cdot 7H_2O$	0.0005
H_3BO_3	0.025
$Na_2MoO_4 \cdot 2H_2O$	0.0001

2.3. Yields and root biomass

At flowering (31 October – 17 November 2020), the daily number of flowers per corm and the yield of the spice were measured. The spice was obtained by dehydrating the stigmas in the shade for 48 - 72 h and then in a cold-dryer (Northwest Technologies NWT100 dryer, Boves, Italy) at 20 °C for 48 h (Vallino et al., 2021).

The roots of each sample collected for AM colonization assessment were weighed fresh. The part of the roots used for the biomass measurement was weighed fresh and then dried in an oven at 60 °C for one week to record the dry biomass. The dry weight of the total roots was calculated by comparing the dry weight of the partial roots with the fresh weights of both the total and partial roots.

During the vegetative phase (end of January 2021), the number of leaves per corm and leaf length were measured on two to three plants per biological replicate, for a total of eight to nine plants per treatment. At the end of the vegetative phase (June 2021), the corms of four Ctr plants per biological replicate (for a total of 12 plants) and of seven to nine inoculated plants per biological replicate (for a total of 21 to 27 plants) were detunicated and the number, fresh weight, and size of the replacement corms were taken. For corm size, the average diameter was calculated after measuring the major and minor diameter of each corm.

2.4. Quality analyses on the saffron spice

2.4.1. Spice extract preparation

Aqueous extracts of the spice were prepared as in Caser et al. (2020). Briefly, 50 mg of ground spice was suspended into 5 mL of deionised water. The solution was stirred (1000 rpm) for 1 h in the dark at room temperature (~21 °C), centrifuged (4 °C and 10,000 rpm), and filtered with PVDF syringe filters (25 mm diameter and 0.45 μm pore size - CPS Analitica, Milan, Italy). Two technical replicates per biological replicate were prepared.

2.4.2. Saffron quality according to ISO 3632 (2011)

The International Standard Organization (ISO 3632) sets the quality standards for the saffron spice. It requires the principal metabolites to be expressed as absorbance readings of 1% (w/v) saffron aqueous extract at 257 (picrocrocin), 330 (safranal), and 440 nm (crocins) using UV–vis spectrophotometry (García-Rodríguez et al., 2017). The saffron extracts were diluted 80x with deionised water and analysed with a spectrophotometer UV–Vis (Cary 60 UV–Vis, Agilent Technologies, Santa Clara, California, USA). The data were related to the dry matter percentage and expressed as the absorbance of a 1% (w/v) spice aqueous solution using 1 cm pathlength cells (A1% 1 cm λ max). The following formula (readapted from Giupponi et al., 2019) was used:

$$A1\%1cm(\lambda max) = (D \times dilxV)/m \times (100 - wMV)$$

where "D" is the specific absorbance, "dil" is the dilution of the extracts, "V" is the volume of the extraction solvent, "m" is the mass in grams of the extracted spice, and "wMV" is the moisture determined using the following formula:

$$wMV = [(m0 - m1) / m0] \times 100$$

where m0 is the mass (g) of the spice before drying and m1 is the mass (g) after drying in an oven for 16 h at 103 ± 2 °C. The analysis was

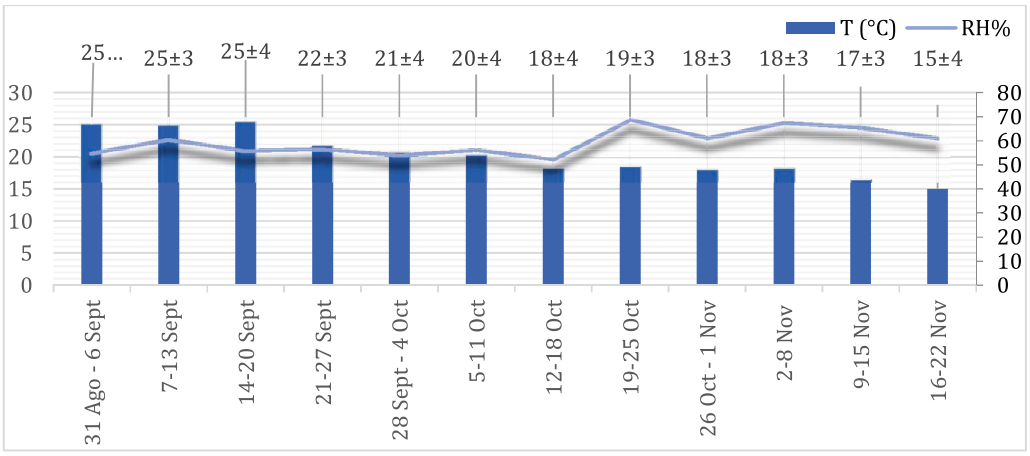

Fig 1. Weekly means and standard deviations of temperature (T, bars) and relative humidity (line) in the greenhouse from 31 August till the end of November.

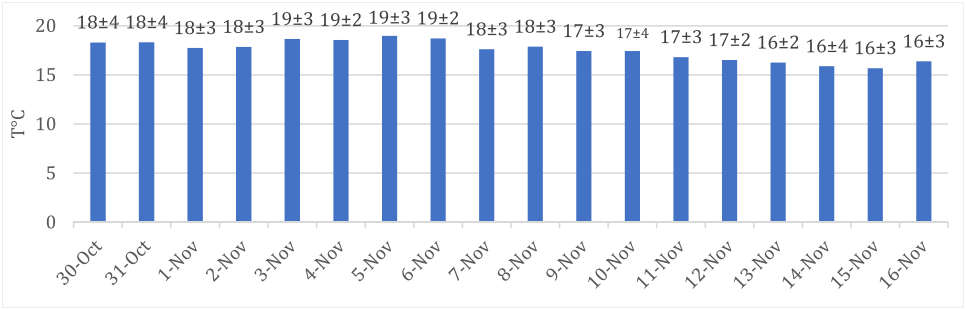

Fig 2. Daily means and standard deviations of temperature (T) in the greenhouse during flowering (30 October – 16 November).

conducted in the dark. The extracts were stored at −20 °C for further analyses.

2.4.3. *Identification and quantification of apocarotenoids by HPLC*

According to García-Rodríguez et al. (2014)water extracts prepared according to ISO 3632 (1:2011) are suitable for determining the safranal content. The concentrations of crocins I and II, i.e., *trans*-crocetin di-(β-D-gentiobiosyl) ester and *trans*-crocetin di-(β-D-glucosyl)-(β-D-gentiobiosyl) ester, and safranal in saffron extracts were detected using an Agilent 1200 High-Performance Liquid Chromatography coupled with an Agilent UV–Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA). Each compound was determined by comparing retention times and UV spectra with those of the standards under the same chromatographic conditions. The standards were crocin I, crocin II, and safranal, purchased from Sigma-Aldrich (Saint Louis, MO, USA). The results were expressed as mg 100 g^{-1} dry weight (DW).

The chromatographic separation was made with a Kinetex C18 column (4.6 × 150 mm^2, 5 μm, Phenomenex, Torrance, CA, USA) and acetonitrile in water as the mobile phase. The chromatographic conditions were 5% to 95% (v/v) acetonitrile in 30 min and 95% to 5% (v/v) acetonitrile in 5 min (10 min conditioning time); flow: 0.6 mL min^{-1}. The detection of crocins and safranal was assessed at 310 nm (Fig. 3).

2.4.4. *Total phenolic content*

The total phenolic content was evaluated with the Folin-Ciocalteu method (Caser et al., 2020). In each tube, 200 μL of spice extract was added to 1000 μL of Folin-Ciocalteu reagent diluted 1:10 with deionised water (v/v). After the solutions were left in the dark at room temperature for 10 min, 800 μL of Na_2CO_3 7.5% (w/v) was put in. After incubation in the dark at room temperature for 30 min, the absorbance at 765 nm was measured by means of a UV–Vis spectrophotometer (Cary 60 UV–Vis, Agilent Technologies, Santa Clara, CA, USA). The data were

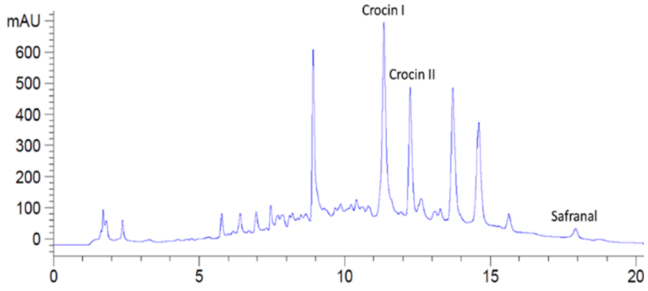

Fig. 3. Chromatogram of saffron water extracts recorded at 310 nm. The following compounds are present: crocin I, crocin II, and safranal.

plotted against a gallic acid calibration curve and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry weight (mg GAE 100 g^{-1} DW).

2.5. *Ecophysiological measurements and determination of pigment content*

During the vegetative phase (end of January 2021) the net CO_2 assimilation rate (A_N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*, mmol H_2O m^{-2} s^{-1}), and leaf transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}) were measured on six plants per treatment with an InfraRed Gas Analyzer (IRGA, ADC, model LCi-Pro; Hoddesdon, UK) (Patono et al., 2022). The leaf chamber of the instrument had a square (6.25 cm^2) aperture sealed around the edge. Saffron has peculiar leaves, which grow from the corm buds and are narrow, long, and pointed. The middle parts in length of three intact, green, healthy leaves per plant were placed for about one minute in the leaf chamber for reading.

Measurements were taken between 1 and 3 pm, the concentration of CO_2 was 344 ± 58.5 ppm and the air pressure 97.2 ± 0.0 kPa. The temperature in the greenhouse ranged from 17 °C to 20 °C. The leaf area (LA, cm^2) was calculated as in Kumar (2009) using the equation [$LA = 191.33e^{(L)0.0037}$], where "L" is the leaf length (mm) (Kumar, 2009).

Fifty mg of fresh leaves from six samples per treatment were then analysed for the content of chlorophylls (chl) and carotenoids according to Lichtenthaler (1987). The leaves were ground in 5 ml of 90% (v/v) methanol in water and, after an over-night extraction at 4 °C in the dark, the pigment concentration was spectrophotometrically (Cary 60 UV–Vis, Agilent Technologies, Santa Clara, CA, USA) determined at 665.2 (chl a), 652.4 (chl b), and 470 (car) nm (Caser et al., 2017; Lichtenthaler, 1987).

2.6. Starch content of the replacement corms

The starch content was analysed in three completely formed replacement corms per treatment using the Megazyme total starch assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). The analysis was based on the procedures for the determination of starch in samples containing resistant starch, D-glucose, and/or maltodextrins and the removal of D-glucose and maltodextrins by alcohol wash (Blandino et al., 2010; Fernandes et al., 2012). The corm moisture content (%) was calculated after drying in an oven at 105 °C for 24 h. The starch content was expressed as % w/w (dry weight basis).

2.7. Statistical analysis

Data were checked for normality (Shapiro–Wilk's test, $p > 0.05$) and homoscedasticity (Levene's test, $p > 0.05$). Significant differences were verified with one-way ANOVA ($p < 0.05$) and Tukey's test. When the ANOVA assumptions were not met, the data were analysed with Kruskal–Wallis test ($p < 0.05$) and Dunn's comparison test with Bonferroni adjustment. The R-studio software was used.

3. Results

3.1. Mycorrhization, bacterial presence, and PGP activities

Alow intraradical presence of the AMF was found in the root fragments, with hyphae and vesicles. Regarding the Myc and Mix treatments, extraradical hyphae of *R. intraradices* were found in 18% (Myc) and 12% (Mix) of root fragments, and intraradical colonization in 15% (Myc) and 10% (Mix) of fragments. For a few fragments of both Myc and Mix treatments, AM colonization ranged from >10% to >90%. AMF were not seen for the Pgpr treatment and uninoculated Ctr.

Main PGP activities and the presence of *Bacillus megaterium* CB97032 and *Paenibacillus durus* CB1806 in the rhizosphere of Ctr, Pgpr, and Mix plants were verified. A differential medium allowed the probable identification of the two bacteria. The bacterial concentration observed in both general (TSA) and free-nitrogen (A6) media for Pgpr and Mix samples was significantly higher than for Ctr samples and not different between the two treatments (Fig. 4). The bacterial concentration in TSA medium was 3.88E+06 ± 6.80E+05 CFU mL^{-1} for Ctr, 2.54E+07 ± 1.07E+07 CFU mL^{-1} for Pgpr, and 3.30E+07 ± 1.80E+07 CFU mL^{-1} for Mix; in A6 medium it was 6.10E+06 ± 1.19E+06 CFU mL^{-1} for Ctr, 3.70E+07 ± 1.94E+07 CFU mL^{-1} for Pgpr, and 3.10E+07 ± 2.00E+06 CFU mL^{-1} for Mix. All PGP activities analysed were significantly higher in samples deriving from Pgpr and Mix treatments than in samples from Ctr, with no differences between the two treatments (Fig. 5). The ammonium (NH_4^+) measured and related to the fixed N_2 potential was 380.73 ± 57.65 mg L^{-1} for Ctr, 726.18 ± 114.11 mg L^{-1} for Pgpr, and 684.36 ± 71.54 mg L^{-1} for Mix. The average radius of the P solubilization halo measured after 7 days was 3.25 ± 0.50 mm for Ctr, 5.75 ± 0.50 mm for Pgpr, and 5.00 ± 0.00 mm for Mix. The average radius of the K solubilization halo measured after 7 days was 8.00 ± 0.00 mm for Ctr, 11.00 ± 1.00 mm for Pgpr, and 11.67 ± 0.58 mm for Mix. The average radius of the Fe^{2+} mobilization halo measured after 7 days and related to siderophore production was 2.75 ± 0.50 mm for Ctr, 4.75 ± 0.50 mm for Pgpr, and 6.00 ± 1.15 mm for Mix.

3.2. Flowering trend, growing degree days, and yields

Flowering lasted from 30 October to 16 November. The anthesis of *C. sativus* began without major differences between the treatments, i.e. 63 (Ctr, Myc, and Mix) or 64 (Pgpr) days after corm sowing (Fig. 6). Flowering lasted 13 (Ctr), 15 (Mix), 17 (Pgpr), or 18 (Myc) days. Plants generated two main flowering peaks on the same days, 69 (19 ± 3 °C) and 72 (18 ± 3 °C) days after sowing. The flower percentages were 25% (Mix), 26% (Ctr), 29% (Pgpr), and 30% (Myc) on November 5th (first flowering peak); 25% (Myc), 30% (Pgpr), 31% (Mix), and 32% (Ctr) on November 8th (second flowering peak).

GDD and flower yield (flowers $corm^{-1}$) were not significantly affected by the treatments (Table 2). Conversely, mg of spice per flower was significantly reduced in plants treated with the AM fungus (Myc) (6.7 ± 1.8 mg) compared with not-inoculated controls (7.6 ± 1.5 mg) and the other inoculated plants, i.e., Pgpr and Mix (Table 2).

Regarding the corm yield (Table 2), Myc treatment gave the highest number of corms (6.9 ± 0.1) and both Myc and Pgpr corms showed lower size than Ctr, but similar weight. Mix plants performed better than Myc for corm weight and both Myc and Pgpr for corm size. When larger corms were considered (> 15 mm), the number of corms was different

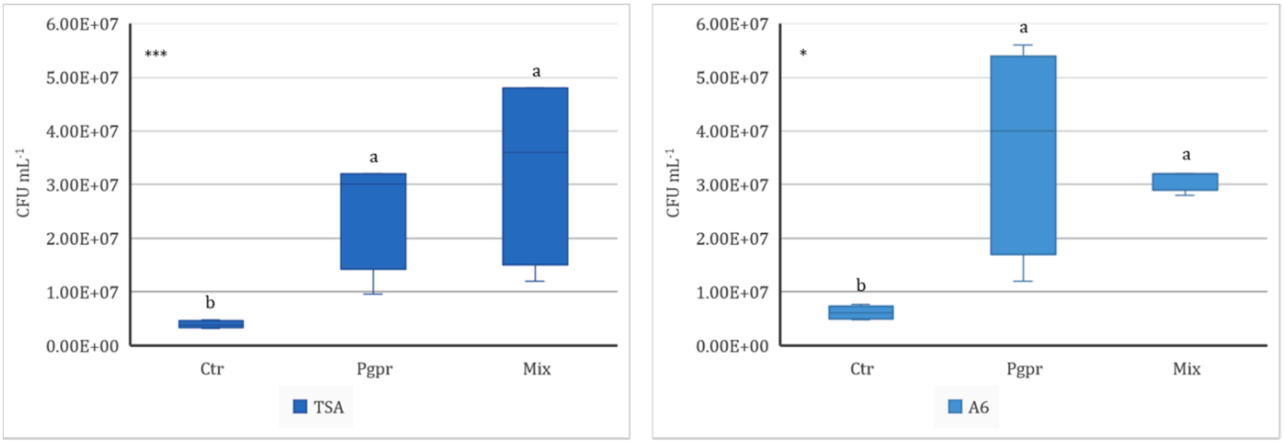

Fig. 4. Bacterial quantification in general (TSA) and free-nitrogen (A6) media of rhizosphere samples from Ctr, Pgpr, and Mix plants. Values with the same letter are not statistically different at $*p < 0.05$ and $***p < 0.001$.

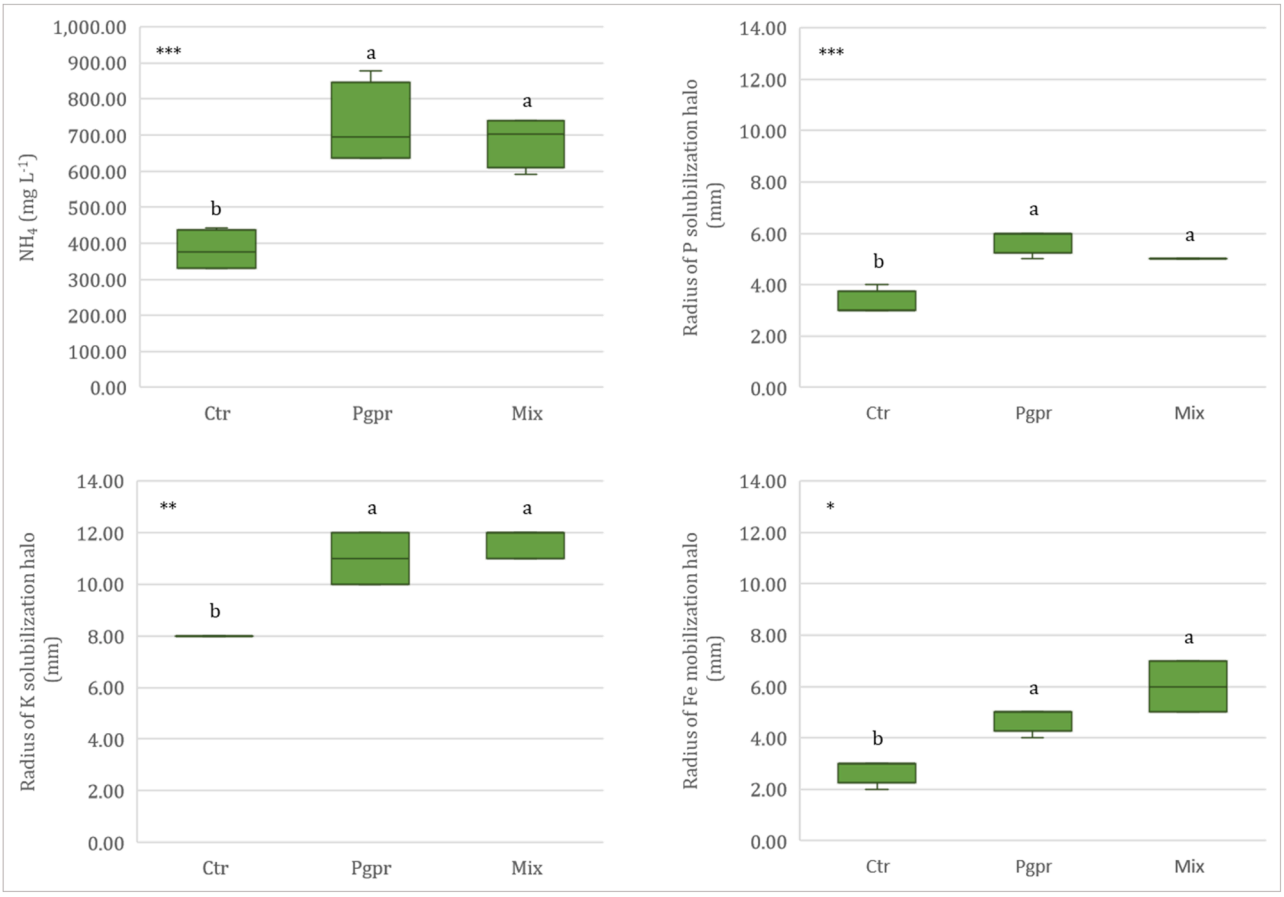

Fig. 5. Ammonium measured with a colorimetric method and related to the fixed N_2 potential; average radius of the P solubilization halo measured after 7 days; average radius of the K solubilization halo measured after 7 days; average radius of the Fe^{2+} mobilization halo measured after 7 days and related to siderophore production. The analyses were performed on rhizosphere samples of Ctr, Pgpr, and Mix plants. Values with the same letter are not statistically different at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

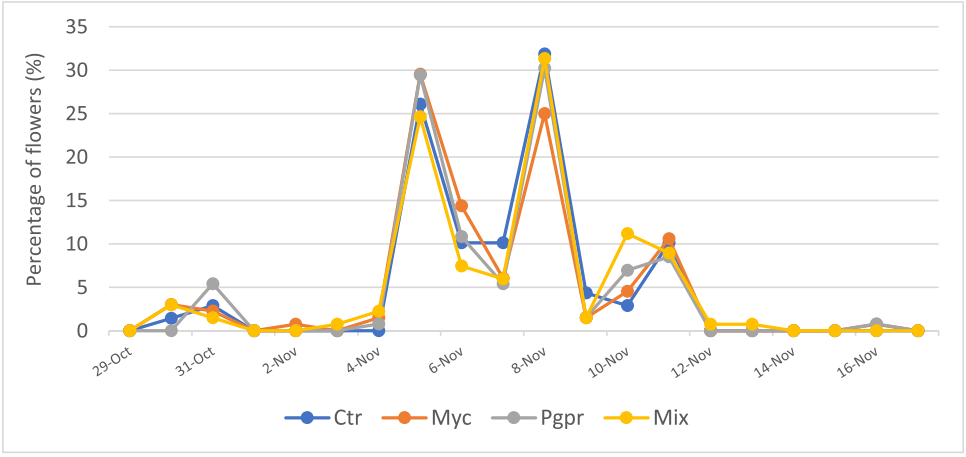

Fig. 6. Percentage of daily harvested flowers of controls (Ctr) and inoculated (Myc, Pgpr, and Mix) saffron plants.

from Ctr only in the case of Pgpr plants, that produced less corms. Mix corms presented a similar number and were the only ones to have a higher weight (4.1 ± 0.6 g) than Ctr.

3.3. Quality analysis on the spice

The spice obtained from all plants had a moisture content below 12% and belonged to the quality category I (ISO 3632, 2011) (Table 3). No differences were seen between treatments for coloring strength (crocins). The flavoring strength (picrocrocin) was significantly reduced in Myc samples compared with Mix. The aromatic strength (safranal) resulted higher in Ctr than in Mix. When the safranal content was analysed by HPLC analysis (Table 3), it was significantly improved in all treated plants compared with controls, which presented 1.3 ± 0.6 mg 100 g^{-1} of safranal.

The total phenolic content (TPC) was significantly higher in the case

Table 2
Growing degree days (GDD), yield of flowers and spice, root biomass (dry weight), and corm yield of controls (Ctr) and inoculated saffron plants (Myc, Pgpr, and Mix).

	Ctr	Myc	Pgpr	Mix	*p*
Flowering					
GDD (°C-days)	877.0 ± 22.9	874.4 ± 21.7	874.6 ± 19.5	875.3 ± 26.4	ns
Flowers corm^{-1} (n)	3.8 ± 0.9	3.7 ± 1.0	3.6 ± 1.2	3.7 ± 1.4	ns
Weight of spice flower^{-1} (mg)	7.6 ± 1.5 a	6.7 ± 1.8 b	7.4 ± 1.8 a	7.5 ± 1.6 a	**
Root biomass (g)	0.49 ± 0.12	0.49 ± 0.07	0.56 ± 0.10	0.62 ± 0.16	ns
Corm yield					
Corms plant^{-1}	6.0 ± 0.0 c	6.9 ± 0.1 a	6.4 ± 0.1 b	6.3 ± 0.1 b	***
Corm weight plant^{-1} (g)	3.4 ± 0.1 ab	3.1 ± 0.1 b	3.4 ± 0.1 a	3.6 ± 0.3 a	*
Corm size plant^{-1} (mm)	19.2 ± 0.7 a	17.7 ± 1.0 b	18.0 ± 0.6 b	18.6 ± 1.3 a	***
Yield of corms with major diameter > 15 mm					
Corms plant^{-1}	6.2 ± 0.3 a	5.8 ± 0.1 a	5.3 ± 0.1 b	5.7 ± 0.1 ab	**
Corm weight plant^{-1} (g)	3.1 ± 0.4 b	3.3 ± 0.1 ab	3.8 ± 0.1 ab	4.1 ± 0.6 a	*
Corm size plant^{-1} (mm)	19.3 ± 0.1 a	18.4 ± 0.1 b	19.5 ± 0.2 a	19.5 ± 0.7 a	*

Values of mean ± standard deviation are reported. Letters indicate statistical differences. Values with the same letter are not statistically different at.
* $p < 0.05$;.
** $p < 0.01$;.
*** $p < 0.001$; ns = not significant.

Table 3
Results of the ISO (3632, 1:2011), total phenolic content (TPC), and HPLC analyses of aqueous extracts of the spice obtained from control (Ctr) and inoculated plants (Myc, Pgpr, and Mix).

ISO (3632, 1:2011)	Ctr	Myc	Pgpr	Mix	*p*
Colour/Crocins $A_1^{1\%}{}_{cm}$ (λ 440)	237.0 ± 17.4 (I)	236.1 ± 17.4 (I)	217.0 ± 33.8 (I)	227.3 ± 6.6 (I)	ns
Flavour/ Picrocrocin $A_1^{1\%}{}_{cm}$ (λ 257)	97.7 ± 6.3 (I) ab	89.9 ± 5.0 (I) b	95.1 ± 4.5 (I) ab	98.0 ± 3.2 (I) a	*
Aroma /Safranal $A_1^{1\%}{}_{cm}$ (λ 330)	36.9 ± 1.8 (I) a	36.7 ± 1.8 (I) ab	31.7 ± 8.7 (I) ab	31.6 ± 1.0 (I) b	*
HPLC (λ 310)					
Safranal (mg 100 g^{-1})	1.3 ± 0.6 b	52.1 ± 15.7 a	36.7 ± 11.6 a	40.7 ± 13.2 a	**
TPC					
Folin-Ciocalteu (mg GAE 100 g^{-1})	2756.4 ± 155.6 b	3133.9 ± 392.9 ab	3241.5 ± 240.7 ab	3396.4 ± 415.9 a	*

The quality category (ISO 3632, 2011) is indicated in brackets. The limits for the quality category I are: picrocrocin >70; safranal 20 - 50; crocins >200. Values of mean ± standard deviation are reported. Letters indicate statistical differences. Values with the same letter are not statistically different at.
* $p < 0.05$;.
** $p < 0.01$.

of Mix plants (3396.4 ± 415.9 mg GAE 100 g^{-1}) compared with controls (2756.4 ± 155.6 mg GAE 100 g^{-1}), which gave a result not statistically different from the other treated plants (Table 3).

Table 4
Results of ecophysiological analysis (A_N = net CO_2 assimilation rate; g_s = stomatal conductance; E = leaf transpiration rate), leaf production, leaf area, content of chlorophylls (chl) and carotenoids of the leaves, and corm starch for controls (Ctr) and inoculated plants (Myc, Pgpr, and Mix).

	Ctr	Myc	Pgpr	Mix	*P*
Ecophysiological analysis					
A_N (μmol CO_2 m^{-2} s^{-1})	6.7 ± 4.2	8.3 ± 2.5	6.4 ± 1.5	9.1 ± 4.3	ns
g_s (mmol H_2O m^{-2} s^{-1})	174.2 ± 23.5 a	112.3 ± 21.3 b	128.2 ± 30.1 b	121.7 ± 17.2 b	***
E (mmol H_2O m^{-2} s^{-1})	3.2 ± 0.4 ab	4.0 ± 0.6 a	3.1 ± 0.7 b	3.9 ± 0.3 ab	*
Leaf production and leaf area					
Leaves plant^{-1}	43.1 ± 16.7	47.7 ± 11.1	46.0 ± 12.2	44.6 ± 14.2	ns
Leaf length plant^{-1} (cm)	37.8 ± 4.8	36.9 ± 4.6	37.9 ± 5.4	36.1 ± 4.5	ns
Leaf area plant^{-1} (cm^{-2})	92.0 ± 16.5	106.7 ± 28.4	101.0 ± 31.3	97.2 ± 30.0	ns
Chlorophylls and Carotenoids in the leaves					
Chl a (μg/mg)	1.0 ± 0.0 ab	0.8 ± 0.2 b	0.9 ± 0.0 ab	1.2 ± 0.1 a	*
Chl b (μg/mg)	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	ns
Chl a+b (μg/mg)	1.4 ± 0.0 ab	1.1 ± 0.2 b	1.2 ± 0.0 b	1.6 ± 0.1 a	**
Carotenoids (μg/mg)	25.4 ± 0.3	21.5 ± 3.4	23.4 ± 0.2	27.0 ± 2.8	ns
Starch analysis					
Starch (g 100^{-1} g)	64.5 ± 4.4	50.9 ± 17.1	61.8 ± 9.5	66.8 ± 14.7	ns
Corm weight plant^{-1} (g)	5.5 ± 0.5	5.7 ± 0.1	5.8 ± 0.1	5.9 ± 0.5	ns
Moisture content (%)	6.4 ± 0.2 b	7.0 ± 0.9 a	6.5 ± 0.2 ab	6.9 ± 0.2 ab	*

Values of mean ± standard deviation are reported. Letters indicate statistical differences. Values with the same letter are not statistically different at.
* $p < 0.05$,.
** $p < 0.01$, and.
*** $p < 0.001$; ns = not significant.

3.4. Ecophysiological analysis, epigeal development, and starch content of corms

Looking at the ecophysiological parameters (Table 4), no significant differences were found for the photosynthetic rate (A_N). Ctr plants had a higher stomatal CO_2 conductance (*gs*, 174.2 ± 23.5 mmol H_2O m^{-2} s^{-1}) and a transpiration rate (E, 3.2 ± 0.4 mmol H_2O m^{-2} s^{-1}) not significantly different from those inoculated. Between the treated plants, E was greater in Myc plants (4.0 ± 0.6 mmol H_2O m^{-2} s^{-1}) than Pgpr, and there were no differences regarding *gs*. Finally, leaf production and leaf area were not affected by the treatments (Table 4).

The content of chl a in Mix plants (1.2 ± 0.1 μg mg^{-1}) was significantly higher than in Myc, but not different than in Pgpr and Ctr. Regarding the content of chl b, no differences were found between inoculated plants and Ctr. Consequently, the sum of chl a and b was significantly greater for the Mix treatment (1.6 ± 0.1 μg mg^{-1}) than for Pgpr (1.2 ± 0.0 μg mg^{-1}) and Myc (1.1 ± 0.2 μg mg^{-1}), but not different form Ctr. Concerning the content of carotenoids, there were no differences between all treatments. The starch content (Table 4), measured in corms of similar weight for all treatments, resulted not significantly different. The moisture content of the corms was significantly higher for the Myc inoculation (7.0%) than for Ctr.

4. Discussion

4.1. How beneficial microorganisms affected flowering and saffron yield

Having a very short life, saffron flowers are collected immediately

and more than 100,000 flowers are needed to produce 1 kg of spice (Caser et al., 2020; Mottaghipisheh et al., 2020). Microorganisms can affect flowering time (Aimo et al., 2010; Caser et al., 2019; Rouphael et al., 2015; Sharaf-Eldin et al., 2008). In Caser et al. (2019), AMF treatments (*R. intraradices* alone and with *F. mosseae*) anticipated the anthesis of soilless saffron in a greenhouse by one week. Application of *B. subtilis* significantly advanced saffron flowering in a greenhouse pot experiment (Sharaf-Eldin et al., 2008). In this study, the anthesis was not affected by the microorganisms and began at the end of October, 63 - 64 days after corm sowing, when the growing degree days (GDD) were about 875.3. Two main flowering peaks occurred about seventy days after sowing, with differences in the percentage distributions of flowers especially for Ctr and Myc (values of 26% and 30% respectively for the first peak and 32% and 25% for the second peak).

Beneficial microorganisms did not affect also the yield of flowers per corm as in Caser et al. (2019). In the present study, the AM colonization evaluated during flowering was low. Overall, little extraradical mycelium and intraradical hyphae with vesicles (specialized storage structures) and very rare arbuscules (functional trade structures) were seen under optical microscope, suggesting that *R. intraradices* might have exhibited a saprophytic behavior during flowering (Azcón-Aguilar et al., 1999; Maiti and Ghosh, 2020). According to Smith and Smith (2011), a negative mycorrhizal growth response can be caused by an imbalanced organic C cost to the plant. This might explain the reduction in spice yield (mg of spice per flower) observed for Myc plants (−12%). In a previous work, *R. intraradices* led to a decrease in spice production in the field (−20% mg of spice per flower; Caser et al., 2019). The low AM colonization and the consequent neutral mycorrhizal growth response might be explained by the biological cycle of *C. sativus* L., that flowers in autumn sixty - ninety days after sowing the corms. Large-sized corms already contain nutrient reserves needed for early growth; for example, the concentration of P and N in corms weighting more than 8 g ranged from 2.41 g kg^{-1} to 2.82 g kg^{-1} (P) and from 12.04 g kg^{-1} to 14.88 g kg^{-1} (N) depending on the mother corm size (Koocheki and Seyyedi, 2015). Thus, the large corms used in this study may have had enough reserve nutrients to support flower development. Well mycorrhized saffron roots were observed after flowering by Aimo et al. (2010), Chamkhi et al. (2018), Lone et al. (2016), and Caser et al. (2019). Accordingly, Lone et al. (2016), monthly estimating the frequency of several AMF species in saffron grown in soil (Kashmir, India) with the Biermann and Lindermann (1981) method, observed that the frequency of colonization in the roots increased from 14.86% in September to 90.24% in March and then decreased. Caser et al. (2019) also found that the intensity of AM colonization (*R. intraradices*) in the whole hydroponic saffron root system analysed with the Trouvelot et al. (1986) method was 71.4% with an arbuscule abundance of 58.9% at the end of the vegetative phase of the first year of cultivation.

The bacteria *Bacillus megaterium* and *Paenibacillus durus,* which occur naturally in saffron rhizosphere, have multiple growth promoting traits (Jami Al-Ahmadi et al., 2017; and Kour et al., 2018). In this study they showed important functional PGP traits, such as N_2 fixation, P solubilization, siderophore production, and K solubilization (Backer et al., 2018; Lobo et al., 2019). Compared with Ctr, the fixed N_2 potential by the microbiota improved by +48% and +44% in the Pgpr and Mix samples, respectively; the P solubilization by +43% (Pgpr) and +35% (Mix); the K solubilization by +27% (Pgpr) and +31% (Mix) times; and the siderophores production by +42% (Pgpr) and +54% (Mix) (Fig. 5). When the bacteria were added to the AMF (Mix inoculum), the frequency of root colonization in some fragments was 90% with abundant vesicles and in more cases an abundant extraradical mycelium was seen, in agreement with Rouphael et al. (2015) reporting that various gram-positive bacteria, such as *Paenibacillus* spp. and *Bacillus* spp., can stimulate AMF branching and colonization. Moreover, in Mix plants the bacteria may have mitigated the initial imbalanced C cost to the plants due to the probable saprophytic behavior of the AMF, resulting in a restored spice yield. A promoting effect on the yield of saffron spice was reported for PGPR inoculants in pot trials, i.e. *Curtobacterium herbarum* Cs10 (Díez-Méndez and Rivas, 2017), *Bacillus* sp. strain D5 (Magotra et al., 2021), and *Bacillus subtilis* FZB24® (Sharaf-Eldin et al., 2008). An increment in the corm size in greenhouses was obtained by treating saffron with *Bacillus* sp. strain D5 (Magotra et al., 2021) or AMF species (*R. intraradices* alone or mixed with *F. mosseae*; Caser et al., 2019b). Since corm size is an important parameter for flowering, the effect of bioinoculants on saffron yield could be evaluated over time in a second-year experiment in a greenhouse, as has already been done in the field (Aimo et al., 2010; Magotra et al., 2021).

4.2. The beneficial microorganisms enhanced the aroma of the spice

AM symbiosis is known to induce changes in plant metabolism (Bianciotto et al., 2018; Kumar et al., 2021; Rouphael et al., 2015), increasing the content of health-promoting compounds such as carotenoids and polyphenols (Bianciotto et al., 2018). The organoleptic properties of the spice produced by both inoculated and uninoculated plants belonged to the quality category I of the ISO 3632 (2011) (the highest), as in Caser et al. (2019). However, the aromatic strength ($A^{1\%}_{1\ cm}$ at λ 330; ISO 3632 – 2011), which is related to the safranal content, was significantly lower for Mix plants. The aromatic strength of saffron, measured with UV–vis spectrophotometry, can be altered by other compounds that can absorb at 330 nm, such as crocins (Fig. 3), interfering with the analysis (García-Rodríguez et al., 2017). García-Rodríguez et al. (2017) observed that the determination of safranal by the UV–vis method gave an overestimation compared with the determination by HPLC, with a range from 3.69 to 8.65 mg 100 mg^{-1} of saffron depending on the area of production. Similarly, in our study the HPLC analysis showed a safranal content not in line with the aroma strength measured with the UV–vis method, being higher in the Myc, Pgpr, and Mix samples (up to +96%) than in the Ctr ones. These results are in agreement with Sharaf-Eldin et al. (2008), where the spice derived from corms drenched with a spore solution of *Bacillus subtilis* FZB24® 14 weeks after sowing had a higher content of picrocrocin (+38.9%), crocetin (+75.3%), and safranal (+8.4%) but a lower level of crocin (−60%) than that derived from uninoculated control corms.

The spice produced from Mix plants also showed a total phenolic content (TPC) significantly higher (+19%) than that obtained from controls. A more positive trend of TPC was also visible for both Myc and Pgpr treatments. Probably the synergy created between the AMF and PGPR led to a more beneficial effect for the plant in agreement with Etesami et al. (2021) and Giovannini et al. (2020). Similarly, in Begum et al. (2022) a mixed inoculant of AMF and PGPR considerably increased tobacco (*Nicotiana tabacum* L.) secondary metabolites such as carotenoids and phenols under drought stress conditions. Thus, the AMF and PGPR influenced saffron secondary metabolism enhancing the production of bioactive compounds even though the roots were little mycorrhized, especially when mixed.

4.3. How beneficial microorganisms affected ecophysiological parameters and corm production

After flowering, growth is mainly supported by leaf photosynthesis, which is maintained high during vegetative development (Renau-Morata et al., 2012). Investigation of saffron ecophysiology during the vegetative phase is limited. Some authors (Moradi et al., 2021; Renau-Morata et al., 2012; Yarami and Sepaskhah, 2015; Zhou et al., 2022) studied the photosynthetic activity of saffron analysing the influence of different factors, such as light intensity and spectra, water stress, planting methods, corm size, and salinity and fertilizer levels, but the influence exerted by beneficial microorganisms has never been evaluated so far. Renau-Morata et al. (2012) obtained values of the net CO_2 assimilation rate (A_N) of plants grown in a greenhouse similar to our study. In the field, the plants showed an A_N similar (Yarami & Sepaskhah, 2015) or higher (20–26 μmol m^{-2} s^{-1} - Renau-Morata et al., 2012)

than ours, perhaps thanks to the more favorable irradiation conditions present in the field than in the greenhouse, especially during winter (Zhou et al., 2022). In other plant species, such as *Solanum lycopersicum* L., *Nicotiana tabacum* L., *Prunus maritima* Marshall, and *Phoenix dactylifera* L., the effects of microbial treatments, i.e., AMF, PGPR, and a mix of both microbial types, on ecophysiological parameters have been recently investigated (Begum et al., 2022; Mannino et al., 2020; Raho et al., 2022; Zai et al., 2021). When treated with bioinoculants (the AMF *Rhizoglomus irregulare*; a combination of the AMF *Claroideoglomus claroideum, Funneliformis caledonium* and *F. geosporum*; a combination of two PGPB strains and a commercial inoculum formed by *Glomus* spp. and bacteria) *Solanum lycopersicum* L. did not significantly change A_N, stomatal conductance (*gs*), and evapotranspiration rate (*E*), but when inoculated with the commercial mixed formulation it reduced the total content of chlorophylls (Mannino et al., 2020). In this study, the total chlorophyll content in inoculated plants did not differ from Ctr, even if a tendency to be higher in Mix plants was observed; among inoculated plants it was higher in Mix plants (1.6 ± 0.1 μg mg^{-1}) than in Myc (1.1 ± 0.2 μg mg^{-1}) and Pgpr (1.2 ± 0.0 μg mg^{-1}) plants. As total chlorophyll content is strongly related to leaf N content (Mannino et al., 2020; Padilla et al., 2018), it can be hypothesized that the mycelium may have ameliorated the substrate structure and retained more N-fixing bacteria in the rhizosphere, leading to an improvement in the mineral nutrient uptake, especially N, by the plants. Also regarding leaf production, leaf area, evapotranspiration rate (*E*), and A_N, no differences were found between treated and uninoculated plants. Only the stomatal conductance (*gs*) differed between treated plants and Ctr (174.2 ± 23.5 mmol H_2O m^{-2} s^{-1}) being lower in treated plants. This might be because the ecophysiological parameters were analysed ten days after the last fertigation. Bioinoculated plants usually decrease the *gs* when are under water limitation allowing for water preservation (Sati et al., 2022), e.g., *Arabidopsis thaliana* (L.) Heynh. with *Azospirillum brasilense* in Cohen et al. (2015) and *Solanum lycopersicum* L. in Mannino et al. (2020) and in Chitarra et al. (2016), which used AMF inocula of *R. intraradices* and *F. mosseae*. Among the inoculated plants, *E* was higher in Myc plants (4.0 ± 0.6 mmol H_2O m^{-2} s^{-1}) than in Pgpr plants (3.1 ± 0.7 mmol H_2O m^{-2} s^{-1}) and not different from Mix plants (3.9 ± 0.3 mmol H_2O m^{-2} s^{-1}). This result is in line with the well-known ability of AMF to improve water status of plants, in agreement with the result of corm moisture content, which tended to be higher for treated corms and was significantly superior for Myc plants ($7.0 \pm 0.9\%$) than for Ctr plants ($6.4 \pm 0.2\%$). Microbial inoculants improved A_N and *E*, and chlorophyll content in *Nicotiana tabacum* L. (the AMF *Glomus versiforme* and the PGPR *Bacillus methylotrophicus*, alone and together - Begum et al., 2022) and *Prunus maritima* Marshall, which also presented a higher *gs* only in AMF and Mix treated plants (the AMF *Funneliformis mosseae* and the phosphate-solubilizing fungus *Apophysomyces spartima* - Zai et al., 2021); and in *Phoenix dactylifera* L. they improved *gs* and pigment content (an AMF consortium of 26 species and a mixture of the PGPR *Bacillus megaterium, Arthrobacter globiformis*, and *Enterobacter ludwigii*, alone and together - Raho et al., 2022).

Similarly to the physiological parameters, no differences emerged also regarding the starch content. However, corm yield resulted overall improved for the inoculated plants. In particular, the number of replacement corms was higher for treated plants than for Ctr, especially for the Myc treatment (+13%), without the weight of the corms being decreased. Corm weight is an important attribute for saffron production as corms need to be above a critical size (1 cm of diameter, ~1.1 g) to flower (Douglas et al., 2014). When only larger corms were considered (> 1.5 cm), Mix plants showed a number of corms similar to Ctr but with an increased weight (+24%). These results are in agreement with previous studies on saffron inoculated with beneficial microorganisms. The number of replacement corms was increased by a consortium of six rhizosphere-isolated bacteria (*Acinetobacteria calcoaceticus, Pseudomonas tremae, Pseudomonas kilonensis, Chryseobacteria elymi, Bacillus aryabhattai, Pseudomonas koreensis*) compared with uninoculated controls (without differences for corm weight) in Ambardar and Vakhlu (2013). Average weight of daughter corms was enhanced by *Bacillus megaterium* in Kour et al. (2018) and by *Bacillus* sp. strain D5 in Magotra et al. (2021).

5. Conclusions

Bioinoculants are considered modern agricultural tools able to reduce chemical application, promote plant defense system and enhance phytochemicals, thus increasing the quality of products. They can attract consumer interest in high-quality and sustainable saffron production. The effects of bipartite (plant–PGPR/AMF) and tripartite (plant–PGPR–AMF) interactions on saffron plants were investigated. Simultaneous inoculations of different beneficial microbes in a controlled environment allowed the responses of saffron to be evaluated under reproducible conditions.

The organoleptic profile of the spice produced in hydroponics belonged to the first ISO (2011) category. Terpenes and phenols have nutraceutical properties and demonstrated pharmacological effects. Their quantification is fundamental to evaluate the quality level of the saffron spice for its use in food and in the pharmaceutical sector and to state the potential efficacy of selected bioinoculants. All beneficial microorganisms led to an increased content of the main aromatic metabolite safranal. Inoculation of the AMF *R. intraradices* decreased spice yield, probably due to a saprophytic behavior exhibited by the fungus during flowering. When the PGPR *B. megaterium* and *P. durus* were added, the yield was restored. Thus, the PGPR and AMF appeared to work in synergy. This cooperation was also seen to improve the total phenolic content of the saffron spice and corm production; indeed, Mix plants produced corms with the same size and weight as the Ctr but with a higher number, differently than single-type inoculant applications.

Taking the results together, we may say that *R. intraradices* and the two bacteria *B. megaterium* and *P. durus* may have stimulated the secondary metabolism of the plants improving quality traits, rather than having acted as biofertilizers enhancing the yield, at least during flowering. This study on the effects of different beneficial microorganisms on saffron during its growth phases can lay the basis for further deepen saffron responses, e.g., at the molecular level.

Funding

This research was funded by the program Interreg V-A Francia Italia Alcotra (Grant No. 1139 “ANTEA – Attività innovative per lo sviluppo della filiera transfrontaliera del fiore edule”; and grant no. 8336 “ANTES - Fiori eduli e piante aromatiche: attività capitalizzazione dei progetti ANTEA ed ESSICA”).

CRediT authorship contribution statement

Stefania Stelluti: Formal analysis, Investigation, Data curation, Writing – original draft. **Matteo Caser:** Investigation, Writing – review & editing. **Sonia Demasi:** Investigation, Writing – review & editing. **Esteban Rodriguez Herrero:** Investigation. **Irene García-González:** Investigation. **Erica Lumini:** Conceptualization, Methodology, Writing – review & editing. **Valeria Bianciotto:** Conceptualization, Methodology, Writing – review & editing. **Valentina Scariot:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank Dario Donno for the HPLC analysis, Davide Lucien Patono for the help with the IRGA instrument, Francesca Vanara for the help with the starch analysis, Walter Gaino for the technical support, and Francesco Berruto for the help in plant maintenance and laboratory analyses.

References

Aimo, S., Gosetti, F., D'Agostino, G., Gamalero, E., Gianotti, V., Bottaro, M., Gennaro, M. C., Berta, G., 2010. Use of arbuscular mycorrhizal fungi and beneficial soil bacteria to improve yield and quality of saffron (Crocus Sativus L.). Acta Hortic 850, 159–164. https://doi.org/10.17660/ActaHortic.2010.850.25.

Ambardar, S., Vakhlu, J., 2013. Plant growth promoting bacteria from Crocus sativus rhizosphere. World J. Microbiol. Biotechnol. 29, 2271–2279. https://doi.org/10.1007/s11274-013-1393-2.

Askari-Khorasgani, O., Pessarakli, M., 2019. Shifting saffron (Crocus sativus L.) culture from traditional farmland to controlled environment (greenhouse) condition to avoid the negative impact of climate changes and increase its productivity. J. Plant Nutr. 42, 2642–2665. https://doi.org/10.1080/01904167.2019.1659348.

Avarseji, Z., Kafi, M., Sabet Teimouri, M., Orooji, K., 2013. Investigation of salinity stress and potassium levels on morphophysiological characteristics of saffron. J. Plant Nutr. 36, 299–310. https://doi.org/10.1080/01904167.2012.739249.

Azcón-Aguilar, C., Bago, B., Barea, J.M., 1999. Saprophytic growth of arbuscular mycorrhizal fungi. In: Varma, A., Hock, B. (Eds.), Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer, Berlin Heidelberg, pp. 391–408. https://doi.org/10.1007/978-3-662-03779-9_16.

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 871, 1–17. https://doi.org/10.3389/fpls.2018.01473.

Begum, N., Wang, L., Ahmad, H., Akhtar, K., Roy, R., Khan, M.I., Zhao, T., 2022. Co-inoculation of arbuscular mycorrhizal fungi and the plant growth-promoting rhizobacteria improve growth and photosynthesis in tobacco under drought stress by up-regulating antioxidant and mineral nutrition metabolism. Microb. Ecol. 83, 971–988. https://doi.org/10.1007/s00248-021-01815-7.

Berruti, A., Lumini, E., Balestrini, R., Bianciotto, V., 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front. Microbiol. 6, 1–13. https://doi.org/10.3389/fmicb.2015.01559.

Bianciotto, V., Victorino, I., Scariot, V., Berruti, A., 2018. Arbuscular mycorrhizal fungi as natural biofertilizers: current role and potential for the horticulture industry. Acta Hortic 1191, 207–216. https://doi.org/10.17660/ActaHortic.2018.1191.29.

Blandino, M., Mancini, M.C., Peila, A., Rolle, L., Vanara, F., Reyneri, A., 2010. Determination ofmaize kernel hardness: comparison of different laboratory tests to predict dry-milling performance. J. Sci. Food Agric. 90, 1870–1878. https://doi.org/10.1002/jsfa.4027.

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V., 2020. Saffron (Crocus sativus L.), the king of spices: an overview. Sci. Hortic. (Amsterdam). 272 https://doi.org/10.1016/j.scienta.2020.109560.

Carmona, M., Zalacain, A., Sánchez, A.M., Novella, J.L., Alonso, G.L., 2006. Crocetin esters, picrocrocin and its related compounds present in Crocus sativus stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS. J. Agric. Food Chem. 54, 973–979. https://doi.org/10.1021/jf052297w.

Caser, M., Demasi, S., Stelluti, S., Donno, D., Scariot, V., 2020. Crocus sativus L. Cultivation in alpine environments: stigmas and tepals as source of bioactive compounds. Agronomy 10, 1473. https://doi.org/10.3390/agronomy10101473.

Caser, M., Demasi, S., Victorino, Í.M.M., Donno, D., Faccio, A., Lumini, E., Bianciotto, V., Scariot, V., 2019. Arbuscular mycorrhizal fungi modulate the crop performance and metabolic profile of saffron in soilless cultivation. Agronomy 9, 232. https://doi.org/10.3390/agronomy9050232.

Caser, M., Lovisolo, C., Scariot, V., 2017. The influence of water stress on growth, ecophysiology and ornamental quality of potted Primula vulgaris 'Heidy' plants. New insights to increase water use efficiency in plant production. Plant Growth Regul 83, 361–373. https://doi.org/10.1007/s10725-017-0301-4.

Caser, M., Victorino, Í.M.M., Demasi, S., Berruti, A., Donno, D., Lumini, E., Bianciotto, V., Scariot, V., 2018. Saffron cultivation in marginal alpine environments: how amf inoculation modulates yield and bioactive compounds. Agronomy 9, 12. https://doi.org/10.3390/agronomy9010012.

Chamkhi, I., Abbas, Y., Tarmoun, K., Aurag, J., Sbabou, L., 2018. Morphological and molecular characterization of arbuscular mycorrhizal fungal communities inhabiting the roots and the soil of saffron (Crocus sativus L.) under different agricultural management practices. Arch. Agron. Soil Sci. 65, 1035–1048. https://doi.org/10.1080/03650340.2018.1548012.

Chen, D., Xing, B., Yi, H., Li, Y., Zheng, B., Wang, Y., Shao, Q., 2020. Effects of different drying methods on appearance, microstructure, bioactive compounds and aroma compounds of saffron (Crocus sativus L.). LWT 120, 108913. https://doi.org/10.1016/j.lwt.2019.108913.

Chialva, M., Lanfranco, L., Guazzotti, G., Santoro, V., Novero, M., Bonfante, P., 2020. Gigaspora margarita and its endobacterium modulate symbiotic marker genes in tomato roots under combined water and nutrient stress. Plants 9, 1–14. https://doi.org/10.3390/plants9070886.

Chitarra, W., Pagliarani, C., Maserti, B., Lumini, E., Siciliano, I., Cascone, P., Schubert, A., Gambino, G., Balestrini, R., Guerrieri, E., 2016. Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol. 171, 1009–1023. https://doi.org/10.1104/pp.16.00307.

Cohen, A.C, Bottini, R., Pontin, M., Berli, F.J., Moreno, D., Boccanlandro, H., Travaglia, C.N., Piccoli, P.N., 2015. *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153 (1), 79–90. https://doi.org/10.1111/ppl.12221.

De Pascale, S., Rouphael, Y., Colla, G., 2017. Plant biostimulants: innovative tool for enhancing plant nutrition in organic farming. Eur. J. Hortic. Sci 82, 277–285. https://doi.org/10.17660/eJHS.2017/82.6.2.

Díez-Méndez, A., Rivas, R., 2017. Improvement of saffron production using Curtobacterium herbarum as a bioinoculant under greenhouse conditions. AIMS Microbiol. 3, 354–364. https://doi.org/10.3934/microbiol.2017.3.354.

Douglas, M.H., Smallfield, B.M., Wallace, A.R., Mcgimpsey, J.A., 2014. Saffron (Crocus sativus L.): the effect of mother corm size on progeny multiplication, flower and stigma production. Sci. Hortic. (Amsterdam). 166, 50–58. https://doi.org/10.1016/j.scienta.2013.12.007.

Etesami, H., Jeong, B.R., Glick, B.R., 2021. Contribution of arbuscular mycorrhizal fungi, phosphate–solubilizing bacteria, and silicon to P uptake by plant. Front. Plant Sci. https://doi.org/10.3389/fpls.2021.699618.

Fernandes, B., Dragone, G., Abreu, A.P., Geada, P., Teixeira, J., Vicente, A., 2012. Starch determination in Chlorella vulgaris-a comparison between acid and enzymatic methods. J. Appl. Phycol. 24, 1203–1208. https://doi.org/10.1007/s10811-011-9761-5.

García-Rodríguez, M.V., López-Córcoles, H., Alonso, G.L., Pappas, C.S., Polissiou, M.G., Tarantilis, P.A., 2017. Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron. Food Chem. 221, 838–843. https://doi.org/10.1016/j.foodchem.2016.11.089.

García-Rodríguez, M.V., Serrano-Díaz, J., Tarantilis, P.A., López-Córcoles, H., Carmona, M., Alonso, G.L., 2014. Determination of saffron quality by high-performance liquid chromatography. J. Agric. Food Chem. 62, 8068–8074. https://doi.org/10.1021/jf5019356.

Genre, A., Lanfranco, L., Perotto, S., Bonfante, P., 2020. Unique and common traits in mycorrhizal symbioses. Nat. Rev. Microbiol. 18, 649–660. https://doi.org/10.1038/s41579-020-0402-3.

Giovannini, L., Palla, M., Agnolucci, M., Avio, L., Sbrana, C., Turrini, A., Giovannetti, M., 2020. Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: research strategies for the selection of the best performing inocula. Agronomy 10, 106. https://doi.org/10.3390/agronomy10010106.

Giupponi, L., Ceciliani, G., Leoni, V., Panseri, S., Pavlovic, R., Lingua, G., Di Filippo, A., Giorgi, A., 2019. Quality traits of saffron produced in Italy: geographical area effect and good practices. J. Appl. Bot. Food Qual. 92, 336–342. https://doi.org/10.5073/JABFQ.2019.092.045.

Gresta, F., Lombardo, G.M., Siracusa, L., Ruberto, G., 2008. Saffron, an alternative crop for sustainable agricultural systems. A review. Agron. Sustain. Dev. 28, 95–112. https://doi.org/10.1051/agro:2007030.

Jami Al-Ahmadi, M., Mohammadi, A., Kohabadi, E.S., 2017. Characterization of Bacteria Isolated from the Saffron (Crocus sativus L.) Rhizosphere. J. Hortic. Res. 25, 5–14. https://doi.org/10.1515/johr-2017-0017.

Khan, M., Hanif, M.A., Ayub, M.A., Jilani, M.I., Shahid Chatha, S.A., 2020. Saffron, in: medicinal plants of South Asia. Elsevier 587–600. https://doi.org/10.1016/B978-0-08-102659-5.00043-4.

Koocheki, A., Seyyedi, S.M., 2015. Relationship between nitrogenand phosphorus use efficiency in saffron (Crocus sativus L.) as affected by mother corm size and fertilization. Ind. Crops Prod. 71, 128–137. https://doi.org/10.1016/j.indcrop.2015.03.085.

Kour, R., Ambardar, S., Vakhlu, J., 2018. Plant growth promoting bacteria associated with corm of Crocus sativus during three growth stages. Lett. Appl. Microbiol. 67, 458–464. https://doi.org/10.1111/lam.13042.

Kumar, R., 2009. Calibration and validation of regression model for non-destructive leaf area estimation of saffron (Crocus sativus L.). Sci. Hortic. (Amsterdam). 122, 142–145. https://doi.org/10.1016/j.scienta.2009.03.019.

Kumar, R., Singh, V., Devi, K., Sharma, M., Singh, M.K., Ahuja, P.S., 2008. State of art of saffron (Crocus sativus L.) agronomy: a comprehensive review. Food Rev. Int. 25, 44–85. https://doi.org/10.1080/87559120802458503.

Kumar, S., Arora, N., Upadhyay, H., 2021. Arbuscular mycorrhizal fungi: source of secondary metabolite production in medicinal plants. New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, pp. 155–164. https://doi.org/10.1016/B978-0-12-821005-5.00011-9.

Lanfranco, L., Fiorilli, V., Gutjahr, C., 2018. Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. New Phytol. 220, 1031–1046. https://doi.org/10.1111/nph.15230.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Lobo, C.B., Juárez Tomás, M.S., Viruel, E., Ferrero, M.A., Lucca, M.E., 2019. Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiol. Res. 219, 12–25. https://doi.org/10.1016/j.micres.2018.10.012.

Lone, R., Shuab, R., Koul, K.K., 2016. AMF association and their effect on metabolite mobilization, mineral nutrition and nitrogen assimilating enzymes in saffron (Crocus sativus) plant. J. Plant Nutr. 39, 1852–1862. https://doi.org/10.1080/01904167.2016.1170850.

Magotra, S., Bhagat, N., Ambardar, S., Ali, T., Hurek, B.R., Hurek, T., Verma, P.K., Vakhlu, J., 2021. Field evaluation of PGP Bacillus sp. strain D5 native to Crocus

sativus, in traditional and non traditional areas, and mining of PGP genes from its genome. Sci. Rep. 11, 1–16. https://doi.org/10.1038/s41598-021-84585-z.
Maiti, S.K., Ghosh, D., 2020. Plant–soil interactions as a restoration tool. Climate Change Soil Interactions. LTD. https://doi.org/10.1016/b978-0-12-818032-7.00024-2.
Mannino, G., Nerva, L., Gritli, T., Novero, M., Fiorilli, V., Bacem, M., Bertea, C.M., Lumini, E., Chitarra, W., Balestrini, R., 2020. Effects of different microbial inocula on tomato tolerance to water deficit. Agronomy 10, 1–18. https://doi.org/10.3390/agronomy10020170.
McMaster, G.S., Wilhelm, W.W., 1997. Growing degree-days: one equation, two interpretations. Agric. For. Meteorol. 87, 291–300. https://doi.org/10.1016/S0168-1923(97)00027-0.
Molina, R.V., Valero, M., Navarro, Y., Guardiola, J.L., García-Luis, A., 2005a. Temperature effects on flower formation in saffron (Crocus sativus L.). Sci. Hortic. (Amsterdam). 103, 361–379. https://doi.org/10.1016/j.scienta.2004.06.005.
Molina, R.V., Valero, M., Navarro, Y., García-Luis, A., Guardiola, J.L., 2005b. Low temperature storage of corms extends the flowering season of saffron (Crocus sativus L.). J. Hortic. Sci. Biotechnol. 80, 319–326. https://doi.org/10.1080/14620316.2005.11511937.
Moradi, S., Kafi, M., Aliniaeifard, S., Salami, S.A., Shokrpour, M., Pedersen, C., Moosavi-Nezhad, M., Wróbel, J., Kalaji, H.M., 2021. Blue Light Improves Photosynthetic Performance and Biomass Partitioning toward Harvestable Organs in Saffron (*Crocus sativus* L.). Cells 10, 1994. https://doi.org/10.3390/cells10081994.
Mottaghipisheh, J., Mahmoodi Sourestani, M., Kiss, T., Horváth, A., Tóth, B., Ayanmanesh, M., Khamushi, A., Csupor, D., 2020. Comprehensive chemotaxonomic analysis of saffron crocus tepal and stamen samples, as raw materials with potential antidepressant activity. J. Pharm. Biomed. Anal. 184, 113183 https://doi.org/10.1016/j.jpba.2020.113183.
Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R. B., 2018. Different Responses of Various Chlorophyll Meters to Increasing Nitrogen Supply in Sweet Pepper. Front. Plant Sci. 9, 1752. https://doi.org/10.3389/fpls.2018.01752.
Pandey, D.K., Kaur, P., Dey, A., 2018. Arbuscular Mycorrhizal Fungi: Effects on Secondary Metabolite Production in Medicinal Plants, in: Fungi and Their Role in Sustainable Development: Current Perspectives. Springer Singapore, Singapore, pp. 507–538. https://doi.org/10.1007/978-981-13-0393-7_28.
Patono, D.L., Eloi Alcatrão, L., Dicembrini, E., Ivaldi, G., Ricauda Aimonino, D., Lovisolo, C., 2022. Technical advances for measurement of gas exchange at the whole plant level: design solutions and prototype tests to carry out shoot and rootzone analyses in plants of different sizes. Plant Sci 326, 111505. https://doi.org/10.1016/j.plantsci.2022.111505.
Qaisrani, M.M., Zaheer, A., Mirza, M.S., Naqqash, T., Qaisrani, T.B., Hanif, M.K., Rasool, G., Malik, K.A., Ullah, S., Jamal, M.S., Mirza, Z., Karim, S., Rasool, M., 2019. A comparative study of bacterial diversity based on culturable and culture-independent techniques in the rhizosphere of maize (Zea mays L.). Saudi J. Biol. Sci. 26, 1344–1351. https://doi.org/10.1016/j.sjbs.2019.03.010.
Raho, O., Boutasknit, A., Anli, M., Ben-Laouane, R., Rahou, Y.A., Ouhaddou, R., Duponnois, R., Douira, A., Modafar, C.El, Meddich, A., 2022. Impact of native biostimulants/biofertilizers and their synergistic interactions on the agro-physiological and biochemical responses of date palm seedlings. Gesunde Pflanz 1053–1069. https://doi.org/10.1007/s10343-022-00668-5.
Renau-Morata, B., Nebauer, S.G., Sánchez, M., Molina, R.V., 2012. Effect of corm size, water stress and cultivation conditions on photosynthesis and biomass partitioning during the vegetative growth of saffron (Crocus sativus L.). Ind. Crops Prod. 39, 40–46. https://doi.org/10.1016/j.indcrop.2012.02.009.
Rouphael, Y., Colla, G., 2020. Editorial: biostimulants in agriculture. Front. Plant Sci. 11, 1–7. https://doi.org/10.3389/fpls.2020.00040.
Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., Pascale, S.De, Bonini, P., Colla, G., 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci. Hortic. (Amsterdam). 196, 91–108. https://doi.org/10.1016/j.scienta.2015.09.002.
Salas, M., del, C., Montero, J.L., Diaz, J.G., Berti, F., Quintero, M.F., Guzmán, M., Orsini, F., 2020. Defining optimal strength of the nutrient solution for soilless cultivation of saffron in the Mediterranean. Agronomy 10, 1311. https://doi.org/10.3390/agronomy10091311.
Sati, D., Pande, V., Pandey, S.C., Samant, M., 2022. Recent Advances in PGPR and Molecular Mechanisms Involved in Drought Stress Resistance. J Soil Sci Plant Nutr 23, 106–124. https://doi.org/10.1007/s42729-021-00724-5.
Sharaf-Eldin, M., Elkholy, S., Fernández, J.-A., Junge, H., Cheetham, R., Guardiola, J., Weathers, P., 2008. Bacillus subtilis FZB24® affects flower quantity and quality of saffron (Crocus sativus). Planta Med. 74, 1316–1320. https://doi.org/10.1055/s-2008-1081293.
Stelluti, S., Caser, M., Demasi, S., Scariot, V., 2021. Sustainable processing of floral bio-residues of saffron (Crocus sativus L.) for valuable biorefinery products. Plants 10 (523). https://doi.org/10.3390/plants10030523.
Tarantilis, P.A., Polissiou, M., Manfait, M., 1994. Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. J. Chromatogr. A 664, 55–61. https://doi.org/10.1016/0021-9673(94)80628-4.
Trouvelot, A., Kough, J., Gianinazzi-Pearson, V., 1986. Mesure Du Taux De Mycorhization VA D'un Système radiculaire. Recherche de Méthodes D'estimation Ayant Une Signification Fonctionnelle. Mycorrhizae Physiol. Genet. Gianinazzi-Pearson, V., Gianinazzi, S., Eds.; INRA Press Paris, Fr. 217–221.
Vallino, M., Faccio, A., Zeppa, G., Dolci, P., Cerutti, E., Zaquini, L., Faoro, F., Balestrini, R., 2021. Impact of drying temperature on tissue anatomy and cellular ultrastructure of different aromatic plant leaves. Plant Biosyst. https://doi.org/10.1080/11263504.2021.1922535, 0, 000.
Victorino, Í.M.M., Voyron, S., Caser, M., Orgiazzi, A., Demasi, S., Berruti, A., Scariot, V., Bianciotto, V., Lumini, E., 2021. Metabarcoding of soil fungal communities associated with alpine field-grown saffron (Crocus sativus l.) inoculated with am fungi. J. Fungi 7, 1–15. https://doi.org/10.3390/jof7010045.
Volpe, V., Chitarra, W., Cascone, P., Volpe, M.G., Bartolini, P., Moneti, G., Pieraccini, G., Di Serio, C., Maserti, B., Guerrieri, E., Balestrini, R., 2018. The association with two different arbuscular mycorrhizal fungi differently affects water stress tolerance in tomato. Front. Plant Sci. 9 https://doi.org/10.3389/fpls.2018.01480.
Walker, C., Schüßler, A., Vincent, B., Cranenbrouck, S., Declerck, S., 2021. Anchoring the species Rhizophagus intraradices (formerly Glomus intraradices). Fungal Syst. Evol. 8, 179–201. https://doi.org/10.3114/fuse.2021.08.14.
Yarami, N., Sepaskhah, A.R., 2015. Saffron response to irrigation water salinity, cow manure and planting method. Agricultural Water Management 150, 57–66. https://doi.org/10.1016/j.agwat.2014.12.004.
Zai, X.M., Fan, J.J., Hao, Z.P., Liu, X.M., Zhang, W.X., 2021. Effect of co-inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing fungi on nutrient uptake and photosynthesis of beach palm under salt stress environment. Sci. Rep. 11, 1–11. https://doi.org/10.1038/s41598-021-84284-9.
Zhou, T., Qiu, X., Zhao, L., Yang, W., Wen, F., Wu, Q., Yan, J., Xu, B., Chen, J., Ma, Y., Pei, J., 2022. Optimal light intensity and quality increased the saffron daughter corm yield by inhibiting the degradation of reserves in mother corms during the reproductive stage. Industrial Crops and Products 176, 114396.
Zouari, I., Salvioli, A., Chialva, M., Novero, M., Miozzi, L., Tenore, G.C., Bagnaresi, P., Bonfante, P., 2014. From root to fruit: rNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. BMC Genomics 15, 221. https://doi.org/10.1186/1471-2164-15-221.