Diversity and bioprospection of fungal endophytic microbiome of *Crocus sativus* L. (saffron)

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Abstract: *Crocus sativus* L. commonly known as saffron is a small geophyte comprising of a subterranean corm, leafy vegetative shoot, and purple-colored flowers. The early evidences of cultivation and utilization of saffron dates back to 2500–1500 BC in Mediterranean regions. *C. sativus* has a triploid genotype which results in abnormal gamete formation and hence it is propagated vegetatively. The medicinal and aromatic property of saffron is due to the apocarotenoids- Crocin and safranal, present in the stigma of flower. The cultivation and production of *C. sativus* is constantly declining worldwide for the last few decades due to various biotic and abiotic factors. One of the most important factors that influence plant health is the endophytic community harbored by the host plant. *C. sativus* harbors a huge diversity of fungal endophytes with significant bioactive potential. The application of microbes of endophytic origin for sustainable cultivation and crop management of saffron are also reported. The endophytic microbes of saffron also yield bioactive natural products for pharmacological and industrial applications.

Keywords: Saffron, Kashmir, apocarotenoids, fungal diversity, sustainable cultivation, microbes

Introduction

Crocus sativus L. is a monocotyledonous species of magnoliophyta class and Iridaceae family. *C. sativus* is a triploid species with basic chromosome number x=8, and this triploid nature results in sexual sterility of *Crocus*. The sterility is due to abnormal gamete formation caused by improper chromosomal segregation during meiosis. Therefore, the propagation of *C. sativus* occurs either by vegetative multiplication of the underground corms/bulbs or by interspecific hybridization (Negbi et al. 1989). *C. sativus* is a small geophyte comprising of a subterranean corm/bulb, leafy vegetative shoot and purple-colored flowers (Fig. 1). *C. sativus* is a perennial plant with its life cycle divided into three distinct phases, viz. dormant phase in which corm development and bud sprouting occurs, followed by flowering stage also known as reproductive or generative phase, and lastly vegetative phase which is characterized by leaf development (Wani et al. 2016). The phenology of *C. sativus* is better adapted to the Mediterranean climate and is similar in all saffron growing countries with the difference in timing of phenological events (Fig. 2). *C. sativus* shows summer dormancy, which is a great ecological adaptation strategy devised by the plant to improve its survival under severe drought conditions. This strategy of saffron is of great ecological significance, particularly in view of the changing climate scenario resulting in increased temperature and frequent drought spells.

The most important part of this plant is the dried red stigma known as Saffron[1], which has been used as a medicinal herb and spice since time immemorial (Javadi et al. 2013; Baba et al. 2015a; Ghaffari et al. 2019). *C. sativus* synthesizes unique set of metabolites known as apocarotenoids[2], which are synthesized in the stigma part of the plant (Ashraf et al. 2015). The apocarotenoids of saffron are crocin, picrocrocin, and safranal which are responsible for color, flavor and aroma of saffron, respectively (Kumar et al. 2009). Owing to its high demand in dye, perfumery and flavoring industries, saffron is one of the most expensive spices in the world and is recognized as *red gold*. The diverse compositions of saffron metabolites contribute an important role in plant adaptation to various stress conditions. Apart from these saffron metabolites are reported to have tremendous therapeutic properties and its pharmacological importance has been appreciated both by the traditional, Avicenna's Canon of Medicine (al-Qanun fi al-tib) as well as modern scientific reports (Bhargava, 2011; Hosseinzadeh and Nassiri-Asl, 2013; Baba et al. 2015a; Ghaffari et al. 2019).

Saffron is primarily cultivated in Iran, Spain, India, Greece, Morocco, Italy, Turkey and France (Fernandez et al. 2004). In India, commercial cultivation of saffron occurs mainly in Kashmir[3]. Saffron is being cultivated in Kashmir since 750 AD. However, the cultivation and productivity of *saffron* is observing a constant decline worldwide including J&K state due to various factors, which is a matter of concern to the saffron growers as well as agricultural scientists (Gresta et al. 2008). It is reported that there was a decrease of 83 % in the area and 72 % in the productivity of saffron in a single

[1] In Kashmir Himalayas saffron is locally known as “Kong” in Kashmiri, and “Zafran” in Urdu.
[2] Apocarotenoids are the degradation products of the carotenoids.
[3] Kashmir is a Himalayan valley in the state of Jammu and Kashmir (J&K) in India.

decade in Kashmir (Wani et al. 2016). This decline in cultivation and productivity of saffron worldwide is primarily attributed to lack of conventional breeding approaches due to its sterile nature, poor agronomic practices and disease management strategies. Apart from these the modern biotechnological approach has also failed to deliver due to lack of established transformation protocols in *C. sativus*. Thus, efforts are being made to understand the biology of the crop and replace the traditional practices of saffron cultivation with modern technology driven cultivation approach. The cultivation of *Crocus* is restricted to specific agro climatic regions with temperate climate and also the vegatative propagation of *Crocus* occurs by means of underground corms. It is expected that the microbiome associated with *Crocus* might have significant influence on the adaptation and functioning of the plant (Wani et al. 2016; Wani et al. 2017). Therefore, plant-microbe relationships are being explored as an effective alternative for sustainable cultivation of *C. sativus*.

Decline in saffron productivity: causes and concerns

C. sativus is an important high value crop plant. The cultivation and production of *Crocus* is constantly declining worldwide since last few decades. The cultivation of saffron is restricted to specific agro climatic regions with temperate climate. The replacement of corms, breaking dormancy, transition from vegetative to reproductive stage, development of floral bud, floral emergence, etc. are all tightly regulated by various environmental factors like temperature, irrigation, sunlight, etc. It is reported that temperature regulates the growth and flowering of *Crocus* by affecting the enzyme activity in plant metabolism. Saffron production does not require much water but it is reported that first irrigation is very important for flower emergence and length of flowering period of saffron. However, the declining trend in saffron production and quality is mainly attributed to poor agronomic practices and disease management together with lack of breeding approaches. *C. sativus* has remained outside the realm of genetic improvement because of its sterile nature. Moreover, biotechnological approaches have failed to deliver, because transformation protocol has not been established so far. There are a few reports where genes involved in the flowering and apocarotenoid biosynthetic pathway have been cloned and characterized (Rubio-Moraga et al. 2004; Frusciante et al. 2014; Baba et al. 2015b). Also a few transcription factors regulating the biosynthesis of these compounds have been identified and cloned (Ashraf et al. 2015). However, none of these genes have been taken forward for transforming *Crocus* for enhanced production of apocarotenoids.

An important aspect for sustainable cultivation of saffron is the adequate production of healthy corms which is extremely important to guarantee flower production. However, the corms in their natural environment are constantly under siege from a multitude of disease-causing organisms including viruses, bacteria, nematodes and especially fungi. Several fungal species belonging to genera *Fusarium, Rhizoctonia, Penicillium, Macrophomina, Aspergillus, Sclerotium, Phoma, Stromatinia, Cochliobolus, Sclerotium, Rhizopus, Porostereum, Talaromyces, Epicocum, etc.* are reported to be associated with saffron diseases (Ahrazem et al. 2010; Wani et al. 2016; Wani et al. 2018). Considerable work has been done on pathogens causing diseases in *C. sativus* worldwide

(Cappelli and Di Minco, 1999; Palmero et al. 2014; Gupta and Vakhlu, 2015; Wani et al. 2016; Wani et al. 2017). Corm rot caused by *Fusarium oxysporum* is the most destructive disease in saffron, causing severe performance losses in most saffron fields (Cappelli, 1994). The symptoms of corm rot include pigmentation, and in later stages of disease tissue desiccation takes place. Infected plants die off early which result in reduction of corm yield, flowering and stigma production. The corm rot disease was first detected in Japan (Yamamoto et al. 1954). Corm rot disease is currently widespread throughout the saffron producing countries, causing substantial yield losses. With a disease incidence of 100 % and severity ranging from 6 to 46%, corm rot disease result in reduced plant growth and yield of saffron in Kashmir as well (Husaini et al. 2010).

To avoid pathogen attack *Crocus* corm has developed several physical and chemical barriers, as well as a system of active defense reactions. Recently a new chitinase, SafchiA isolated from corms of *C. sativus* is reported to play an important role in saffron defense response induced by fungal (*Fusarium oxysporum* f. sp. *tuberose*) infection, and mediates inhibition of fungal growth under in-vitro condition (Lopez and Gomez-Gomez, 2009). *C. sativus* is characterized by the presence of saponins in stigma and corm tissues, where they seem to play an antifungal role. The ability of a plant to resist diseases is also dependent on soil conditions such as structure, compaction, drainage, temperature and level of biological activity, along with cultural practices such as planting date and application of fertilizers or herbicides (Ahrazem et al. 2010). In Kashmir valley the commonly used fungicides against *Crocus* corm rot disease are Carbendazim, Myclobutanil, Mancozeb, Bavistin (50 % WP Carbendazim), and Tecto (benzimidazole fungicide). The increased use of chemical fungicides/pesticides to manage diseases caused by various pathogenic agents like fungi, bacteria, actinomycetes, etc. is having a deleterious impact on the natural environment as well as human beings. These chemicals also affect the beneficial microflora associated with the plant and put selection pressure for evolution of resistant pathotypes. Therefore, biological control is gaining importance for integrated pest/ disease management. There is a diverse community of microorganisms (endophytes) which interact positively with plants in agricultural systems in relation to their nutrition and ability to resist biotic and abiotic stress. The endophytes have the potential to be manipulated such that the benefits of their positive effects are harnessed.

Plant – microbe association in *C. sativus*

A lot of work is being done on *C. sativus* to understand the biology of the plant. However, work on plant-microbe interaction in *Crocus* is gaining momentum since last few years. There are various reports of the application of microbes with established plant growth promoting properties on the production of saffron. The antagonistic potential of *Trichoderma viride* isolates collected from soil was investigated against *Crocus* corm rot pathogen, *F. oxysporum* (Mir et al. 2011). Similarly, *Burkholderia gladioli* BG-E39 inhibited the growth of corm rot pathogen *F. oxysporum* and also induced resistance to *Crocus* corm rot disease by priming the plant immune system by modulating the expression of genes involved in various defense mechanisms (Ahmad et al. 2021a). In Spain, the

application of *B. subtilis* FZB24 spore solution to saffron corms significantly increased leaf length, flower per corm, total stigma biomass and decreased the time required for corms to sprout. Moreover, significant increase in the quantity of picrocrocin, crocetin and safranal compounds is reported, when the plants are soil drenched with *B.subtilis* FZB24 spore solution fourteen weeks after the sowing (Sharaf-Eldin et al. 2008). Aytekin and Acikgoz (2008) reported that the production of saffron can be increased by treatment of corms with a synthetic hormone (polystimulin A6 and K) and microorganism based material like bio-humus.

The diversity of bacterial endophytes and rhizospheric bacterial associates of *C. sativus* are reported by culture dependent and culture independent approach (Ambardar and Vakhlu, 2013; Ambardar et al. 2014; Sharma et al. 2015; Ahmad et al. 2021b; Jan et al. 2021). A *Bacillus amyloliquefaciens* strain W2 collected from rhizospheric soil was found effective against corm rot caused by *F. oxysporum* (Gupta and Vakhlu, 2015). Sharma and colleagues isolated cultivable bacterial endophytes from saffron plant and assessed for plant growth promoting activities. Molecular and phylogenetic analysis grouped the fifty-four bacterial isolates into eleven different taxa, viz. *Bacillus licheniformis*, *B. subtilis*, *B. cereus*, *B. humi*, *B. pumilus*, *Paenibacillus elgii*, *B. safensis*, *Brevibacillus* sp., *Pseudomonas putida*, *Staphylococcus hominis* and *Enterobacter cloacae*. *B. licheniformis* was the dominant endophyte in both leaves and corms of saffron. Ambardar and colleagues reported the bacteria associated with rhizosphere, cormosphere and bulk soil of Saffron, using cultivation independent 16S rRNA gene targeted metagenomic approach. Saffron during flowering stage revealed presence of 22 genera but none of the genus was common in all the three samples. Bulk soil bacterial community was represented by 13 genera with *Acidobacteria* being dominant genus, while as rhizospheric bacterial community was represented by eight different genera with *Pseudomonas* being the dominant genus, and Cormospheric bacterial community comprised of six different genera, dominated by the genus *Pantoea* (Ambardar et al. 2014). Ahmad and colleagues isolated 47 bacterial endophytes belonging to 28 genera from *C. sativus* (Ahmad et al. 2021b). The bacterial endophytic community was dominated by the genus *Bacillus*, followed by *Burkholderia* and *Pantoea* with an isolation frequency of 21.6%, 18.6%, and 6.5%, respectively (Ahmad et al. 2021b). Ahmad et al. reported that several bacterial endophytes showed significant plant growth promoting potential and few endophytes were found to inhibit the growth of plant pathogens as well. In another study, Ahmad and colleagues isolated an antibacterial compound Juglomycin A, from a bacterial endophyte *Streptomyces achromogenes*, isolated from *C. sativus* (Ahmad et al. 2020).

Although the accessions of *C. sativus* cultivated in different regions show little genetic variability, the yield and productivity of saffron vary considerably. This could be attributed to variations in agricultural practices, and various biotic and abiotic factors. One of the most important factors that influence plant health is the endophytic community harbored by the host plant. The cultivation of *Crocus* is restricted to specific agro climatic regions with temperate climate and also the propagation of *Crocus* is by means of underground corms. Therefore, the microbiome associated with *Crocus*

might have significant influence on the adaptation and functioning of the plant. Thus, it is imperative to understand the patterns of distribution and community structure of endophytes of *C. sativus*, as well as their interactions with the host plant, for sustainable agriculture and crop management of this high value medicinal and aromatic plant.

Fungal endophytic microbiome of *C. sativus*

A lot of research has been conducted on the diversity of endophytes associated with *C. sativus* and it is found that *C. sativus* harbors a huge diversity of fungal and bacterial endophytes (Wani et al. 2016; Ahmad et al 2021b; Jan et al. 2021). However, in this chapter our focus is only the fungal endophytes of *C. sativus*. Wani et al. isolated 294 fungal endophytic microorganisms from the corms of *C. sativus* cultivated in J&K, India. On the basis of phenotypic characters like growth pattern, colony texture and colony color, as well as morphology of conidia and conidiophores, these 294 fungal endophytic isolates were grouped into 100 morphotypes. Molecular phylogenetic studies based on ITS1-5.8S-ITS2 ribosomal gene sequence analyses assigned these endophytes into 36 distinct species, spreading over 19 genera (Table 1). In another study, Jan et al. isolated 1170 endophytic microorganisms from different plant organs including vegetative and floral organs (Jan et al. 2021). Molecular phylogeny assigned these endophytic isolates to 84 operational taxonomic units (OTUs) spreading over 15 genera, with 7 genera each from bacteria and fungi and one actinomycete genus. The diversity and composition of the endophytic community was almost similar across different sites in J&K state. However, the diversity and composition of the endophytic community varied temporally at the two different phenological stages of *Crocus* lifecycle. It was higher at the dormant than at the vegetative stage, indicating influence of host/corm health status on the fungal endophytic diversity (Fig. 3). This may be explained by the fact that the nutritional and health status of the saffron corms change during the vegetative stage which is less supportive for microbial growth or support the growth of fewer endophytes inside the corm tissues. In addition, the winter season during vegetative stage is marked by snowfall and low temperatures, thus creating conditions that are less favorable for the growth of the endophytes (Wani et al. 2016). It is interesting to note that the fungal endophytic community associated with *C. sativus* in the above two studies showed variation. Wani et al. reported that the dark septate endophytes (DSEs) dominate the saffron endophytic microbiome with an isolation frequency of more than 30%, particularly *Phialophora mustea* and *Cadophora malorum* being the most dominant endophytes (Table. 1). However, Jan et al. reported that *Aspergillus ustus* and *Talaromyces pinophilus* were the most abundant fungal endophytes in saffron, although they recovered dark septate endophytes but in less abundance. This variation in endophytic community composition of *C. sativus* collected from same region demands for a more holistic study in which all the tissues types of saffron at different phenological stages are used for endophyte isolation to unravel the core microbiota of *C. sativus*.

C. sativus is cultivated in water deficient conditions and the association of DSEs with saffron is of great ecological significance, as it is reported that the melanized hyphae[4] of DSEs are important for the host to survive water stress conditions. The melanin pigment in the cell walls of fungal hyphae of DSEs can trap and eliminate oxygen radicals generated during abiotic stress, particularly drought stress. The phylogenetic arrangement of all the four species of DSEs isolated from *C. sativus* into a single clad infers the influence of host plant genotype on selective recruitment of endophytes (Fig. 4). This also indicates host-endophyte specificity in the *Crocus* plant vis-à-vis *P. mustea* and *C. malorum,* and these species being the most preferred endophytes of the host plant. It is suggested that the associations between *Crocus* and DSEs might have developed over centuries and the DSEs being vertically transmitted through vegetative propagation of *Crocus* corms as systemic endophytes of *C. sativus* (Wani et al. 2016). It is reported that the production of phytohormones, particularly Indole Acetic Acid (IAA), increases colonization efficiency of the endophytes by interfering with the host defense system (Navarro et al. 2006). Most of the fungal endophytes of *C. sativus,* including the DSEs produce IAA in significant amount (Wani et al. 2016). Therefore, the fungal endophytes, *P. mustea* and *C. malorum* are efficient colonizers in *C. sativus* and may confer tolerance to the host against a variety of environmental stress factors. It is also reported that among the DSEs isolated from *C. sativus*, *P. mustea* and *C. malorum* isolates showed intra-specific strain variations, thereby suggesting the symbiotic associations between *Crocus* and DSEs are species specific rather than strain specific (Wani et al. 2016).

Some fungal endophytic strains recovered from the *Crocus* corm were identified as being members of commonly observed genera of soil fungi e.g., *Fusarium*, *Penicillium*, *Talaromyces*, *Trichoderma*, and *Paecilomyces*. These fungi are characteristically free-living saprophytes that can also be opportunistic root endophytes or latent pathogens[5]. Pathogenicity assay indicated some of the endophytes of *Crocus* as latent pathogens, as they displayed virulence with varying levels of severity under both *in vitro* and *in vivo* conditions (Fig. 5). For instance, endophytes like, *Alternaria alternata*, *Epicoccum nigrum, Fusarium oxysporum*, *Acremonium* sp. *Penicillium pinophilum, Talaromyces cellulolyticus* displayed moderate to high virulence under both *in vitro* and *in vivo* conditions. However, *Aspergillus pseudodeflectus*, *Botrytis fabiopsis*, *Penicillium canescens, Porostereum* sp*., Paecilomyces marquandii, Talaromyces pinophilus* and *Talaromyces verruculosus* displayed low virulence under *In vivo* condition and therefore considered as low risk pathogens (Wani et al. 2016).

Chamkhi et al. studied the fugal endophytic diversity of *C. sativus* cultivated in Taliouine (Morocco). A total of 60 fungal isolates were recovered from segments of *C. sativus* corms and it was observed that *Rhizopus oryzae* was the most dominant fungal endophyte with an isolation frequency

[4] Melanized hyphae are a characteristic feature of dark septate endophytes, as they have melanin pigment present in their hyphae.

[5] They live as normal endophytes in the host plant, but can turn pathogenic under stress condition or produce disease symptom in the host plant upon re-infection.

of 93.4%, followed by *Aspergillus fumigatiaffinis* and *A. niger,* with isolation frequencies of 4.83% and 1.61%, respectively (Chamkhi et al. 2018). Another study on endophytic diversity of *C. sativus* was conducted by Jan et al. (2021) in which 440 fungal endophytic isolates were recovered from different tissue types of *C. sativus* which were grouped into 130 morphotypes based on the morphological characters (Jan et al. 2021). Molecular phylogeny based on ITS rDNA gene sequencing assigned 130 morphotypes into 25 fungal species spreading over 15 genera. In this study all the fungal endophytes belonged to Ascomycota lineage with *Aspergillus, Talaromyces,* and *Fusarium* being the dominant fungal endophytes. Interestingly, the dominant fungal endophytes associated with saffron in all the above studies were not similar and it is suggested that differences in environment of the study areas, selection of tissue types (explants) for endophyte isolation, and phenological stage of the plant organ/explant at the time of collection, might have influenced the variation in endophytic community composition. Plants are confronted with varied environmental challenges at different phenological stages and growing in different geographical regions. These environmental cues in combinatorial effect with host plant genotype shape the endophytic diversity harbored by the host plants (Arnold, 2007; Wani et al. 2015).

Bioactive potential of fungal endophytes of *C. sativus*

The potential of endophytic microorganisms as proficient producers of bioactive natural products and drug-like molecules has received much attention since 1980s. Endophytic microorganisms represent a huge bio-resource for the isolation of novel bioactive natural products for applications in medicine, agriculture, and industry (Porras-Alfaro and Bayman, 2011; Jalgaonwala et al. 2017). This is not surprising in the light of their evolution over millions of years in diverse ecological niches and natural habitats. There are various reports on the promising potential of metabolic extracts from several endophytes of *Crocus* as biofertilizers, antimicrobial and anti-cancerous agents (Nalli et al. 2015; Wani et al. 2016; Chamkhi et al. 2018). Nalli et al. isolated and characterized four new metabolites, Phialomustin A-D from an endophytic fungus *Phialophora mustea* CS7E2 isolated from *C. sativus*. It is reported that Phialomustin B showed potent cytotoxic activity against the human breast cancer cell line T47D with an IC_{50} of 1 µM, Whereas Phialomustin C and D exhibited significant antifungal activity with IC_{50} values of 14.3 and 73.6 µM, respectively, against *Candida albicans* (Nalli et al. 2015). Zheng et al. reported a unique quinazoline alkaloid isolated a fungal endophyte *Penicillium vinaceum,* which was isolated from corms of *C. sativus*, showed significant antifungal activity against *C. neoformans*, *C. albicans* and *T. rubrum* with MIC_{80} values at 16µg/mL, 32µg/mL and 64µg/mL, respectively. Zheng et al. also reported that this compound exhibited moderate cytotoxity against human lung tumor cell line A549, human colon tumor cell line LOVO and human breast adenocarcinoma cell line MCF-7 with IC_{50} values of 76.83µg/mL, 68.08µg/mL and 40.55µg/mL, respectively (Zheng et al. 2012). Another study by Chamkhi et al. reported significant antibacterial and antioxidant properties of fungal endophytes isolated from saffron. The ethyl acetate extracts of *Rhizopus oryzae* was the most bactericidal

followed by *A. fumigatiaffinis* and *A. niger*, whereas ethyl acetate extracts of *A. niger* and *R. oryzae* showed strong antioxidant activity. However, the antioxidant activity of fungal endophytes varied depending upon the method used and solvent system used for the extraction process (Chamkhi et al. 2018).

It has been reported by various studies that most of the fungal endophytes show antagonistic activity against phytopathogens, thereby indicating a strong bio-control potential. Such fungal endophytes having strong bio control potential can be utilized/harnessed to control various plant diseases, like corm rot and other microbial diseases after carrying out further studies under *in-vitro*, *in-vivo* and field conditions. By virtue of the antimicrobial properties, the endophytes may be imparting resistance/tolerance to the host plant against various pathogenic microbes and microbial diseases. The antimicrobial/antagonistic properties of endophytes may be helping them to dominate or influence the microbial populations in the corresponding ecological niches leading to their efficient colonization in the plants. Endophytes are also reported to produce phytohormones like Auxins, Gibberellins, Jasmonic acid, etc. in significant quantities and thereby provide the host plant with added advantage. The production of phytohormones by endophytes has been well reported and the mechanism of action of the phytohormones in plant growth promotion leading to changes in plants morphological and architectural structure, which contributes to the overall growth and development of the plant is elucidated quite well (Wani et al. 2017; Ahmad et al. 2021b).

An oleaginous fungal endophyte, *M. alpina* CS10E4 isolated from *C. sativus* produces polyunsaturated fatty acids (PUFAs) including arachidonic acid (AA). It is reported that *M. alpina* CS10E4 shifts the metabolic flux of *C. sativus* towards enhanced production of apocarotenoids viz, crocin and safranal, by modulating the expression of key genes of apocarotenoid biosynthetic pathway, like Phytoene synthase (PS), Phytoene desaturase (PDS), Beta carotene hydroxylase (BCH), and carotene cleavage dioxygenases (CCDs). Further, *M. alpina* CS10E4 enhanced tolerance to corm rot disease by releasing arachidonic acid, which acts as conserved defense signal and induces jasmonic acid production in endophyte treated *Crocus* corms (Wani et al. 2017). In another study Wani et. al. reported a fungal endophytic basidiomycete, *Porostereum* sp. CSE26 produces chlorinated aromatic compounds (CAMs) i.e., 3-Chloro-4-methoxybenzaldehyde and 2, 3-Dichlorophenyl isothiocyanate, having phytotoxic activity against *Arabidopsis* plants. In a previous study, *Porostereum* sp. CSE26 was reported as a latent pathogen of *C. sativus* (Wani et al. 2016). Therefore, it is presumed that these CAMS may be acting as pathogenic determinants of *Porostereum* sp. CSE26 (Wani et al. 2018).

The fungal endophytes associated with *C. sativus* produce diverse array of bio-molecules like, phytohormone, enzymes, anticancer, antimicrobial, antioxidant, and phytotoxic compounds, etc. Therefore, the fungal endophytes of *C. sativus,* can be harnessed to develop agro-technologies for sustainable cultivation of Saffron and also yield bioactive natural products for pharmacological and industrial applications.

Conclusion

C. sativus is an important medicinal and aromatic plant growing in Mediterranean climate. It is the only plant species which produces apocarotenoids like crocin, picrocrocin and safranal in significant quantity. The apocarotenoids of saffron is known to impart organoleptic properties to saffron making it world's costliest spice. This plant has remained outside the realm of genetic improvement because of its triploid genotype and also the poor agronomic practices and disease management has led to declining trend in saffron cultivation and productivity. Therefore, the need arises to explore other possibilities for enhancing the production of *Crocus* apocarotenoids. The plant-endophyte interface provides an important ecological marketplace for harnessing the potential of endophytes to produce compounds of therapeutic potential or exert their positive influence on plants to enhance the production of specialized metabolites of plant origin. *C. sativus* harbors a great diversity of fungal and bacterial endophytes. These endophytes produce a diverse array of bioactive molecules which can be harnessed for pharmacological and industrial applications.

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Figure legends

Figure 1. Morphology of *C. sativus* L.

Figure 2. Phenology of *C. sativus* L

Figure 3. Diversity profile graph of fungal endophytes at two stages of *Crocus* life cycle. Black line indicates diversity profile at dormant stage while as Red line indicates the diversity profile at vegetative stage. The plot indicates clearly that the diversity of endophytes associated with *C. sativus* is higher during the dormant stage of its life cycle.

Figure 4. Phylogeny of endophytes of *Crocus sativus* using maximum parsimony analysis based on ITS1-5.8S-ITS2 sequence. Only strain names with accession numbers are provided in the phylogenetic tree for the endophytes isolated in this study. The tree is rooted with *Rhizopus microsporus* (a zygomycete, EU798703).

Figure 5. Corms of *C. sativus* re-infected with endophytes produced rotting symptoms with different levels of severity**.**

Tables

Table 1. The table presents the thirty six different fungal endophytes (their GenBank accession numbers and isolation frequency) isolated from *C. sativus*,

ITS genotype	Molecular identification (GenBank accession no.)	Isolation frequency (%)
ITS1	*Aspergillus flavipes* (KR135119)	3.7
ITS2	*Trichoderma harzianum* (KR135120)	3.4
ITS3	*Cadophora malorum* (KR135121)	12.9
ITS4	*Fusarium oxysporum* (KR135122)	4.1
ITS5	*Alternaria alternata* (KR135123)	4.4
ITS6	*Penicillium pinophilum* (KR135124)	3.7
ITS7	*Paecilomyces tenuis* (KR135125)	1.7
ITS8	*Porostereum* sp. (KR135126)	1
ITS9	*Talaromyces pinophilus* (KR135127)	0.3
ITS10	*Aspergillus dimorphicus* (KR135128)	1
ITS11	*Aspergillus terreus* (KR135129)	1.4
ITS12	*Aspergillus iizukae* (KR135130)	1.4
ITS13	*Aspergillus pseudodeflectus* (KR135131)	3.7
ITS14	*Fusarium incarnatum* (KR135132)	0.3
ITS15	*Alternaria* sp. (KR135133)	0.3
ITS16	*Fusarium solani* (KR135134)	1.4
ITS17	*Talaromyces verruculosus* (KR135135)	2.4
ITS18	*Eucasphaeria* sp. (KR135136)	1.7
ITS19	*Penicillium canescens* (KR135137)	2
ITS20	*Talaromyces cellulolyticus* (KR135138)	9.5
ITS21	*Penicillium* sp. (KR135139)	0.7
ITS22	*Penicillium chrysogenum* (KR135140)	0.7
ITS23	*Epicoccum nigrum* (KR135141)	1
ITS24	*Phialophora mustea* (KR135142)	15
ITS25	*Penicillium griseofulvum* (KR135143)	9.2
ITS26	*Ilyonectria robusta* (KR135144)	0.3
ITS27	*Alternaria brassicae* (KR135145)	0.3
ITS28	*Mortierella alpina* (KR135146)	2
ITS29	*Penicillium* sp. (KR135147)	1
ITS30	*Acremonium* sp. (KR135148)	2.4
ITS31	*Cladosporium silenes* (KR135149)	1
ITS32	*Fusarium tricinctum* (KR135150)	1.7
ITS33	*Leptodontidium orchidicola* (KR135151)	2.4
ITS34	*Botrytis fabiopsis* (KR135152)	0.3
ITS35	*Paecilomyces marquandii* (KR135153)	0.3
ITS36	*Gloeosporium* sp. (KR135154)	1

Figures

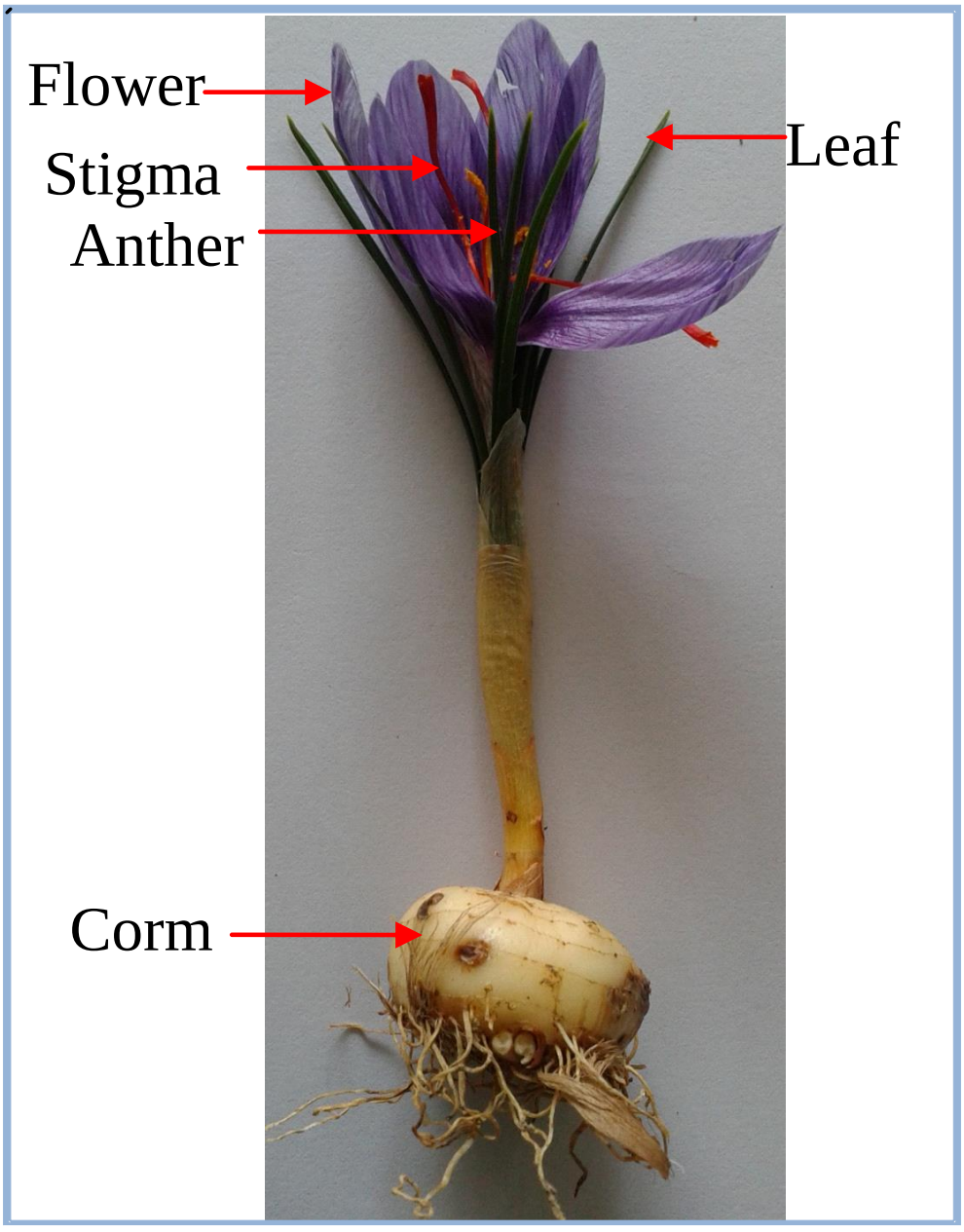


Fig. 1

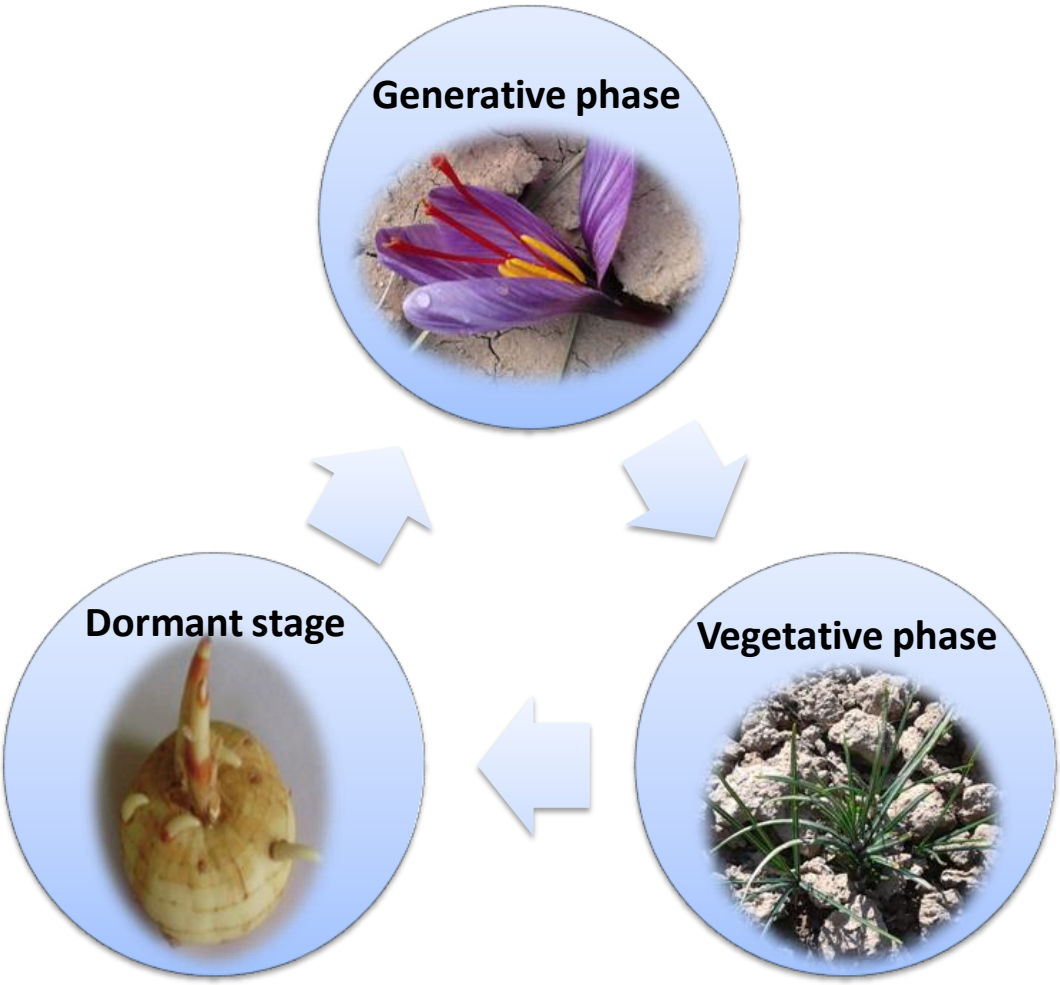
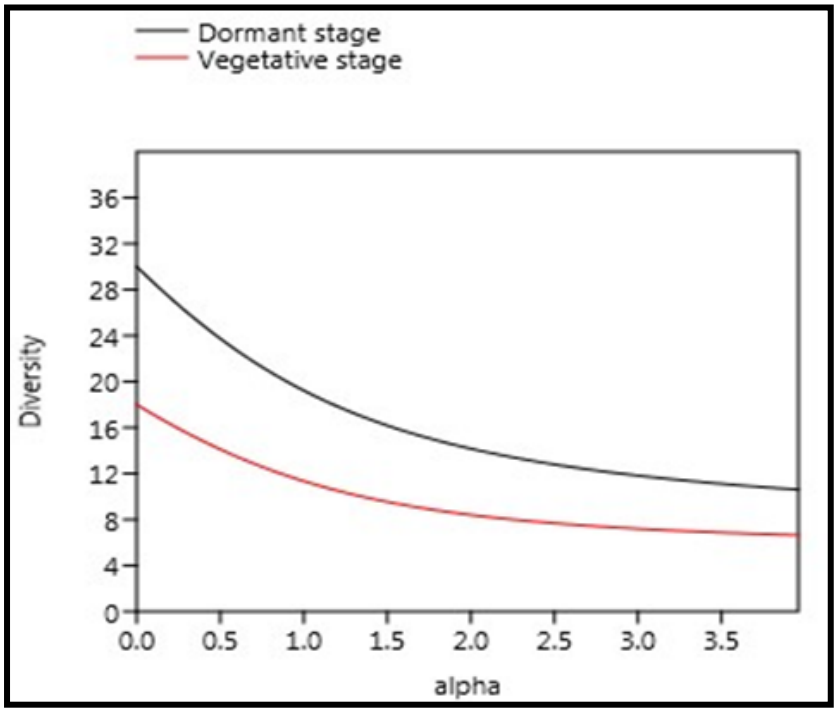


Fig. 2


Dormant stage
Vegetative stage
Diversity
36
32
28
24
20
16
12
8
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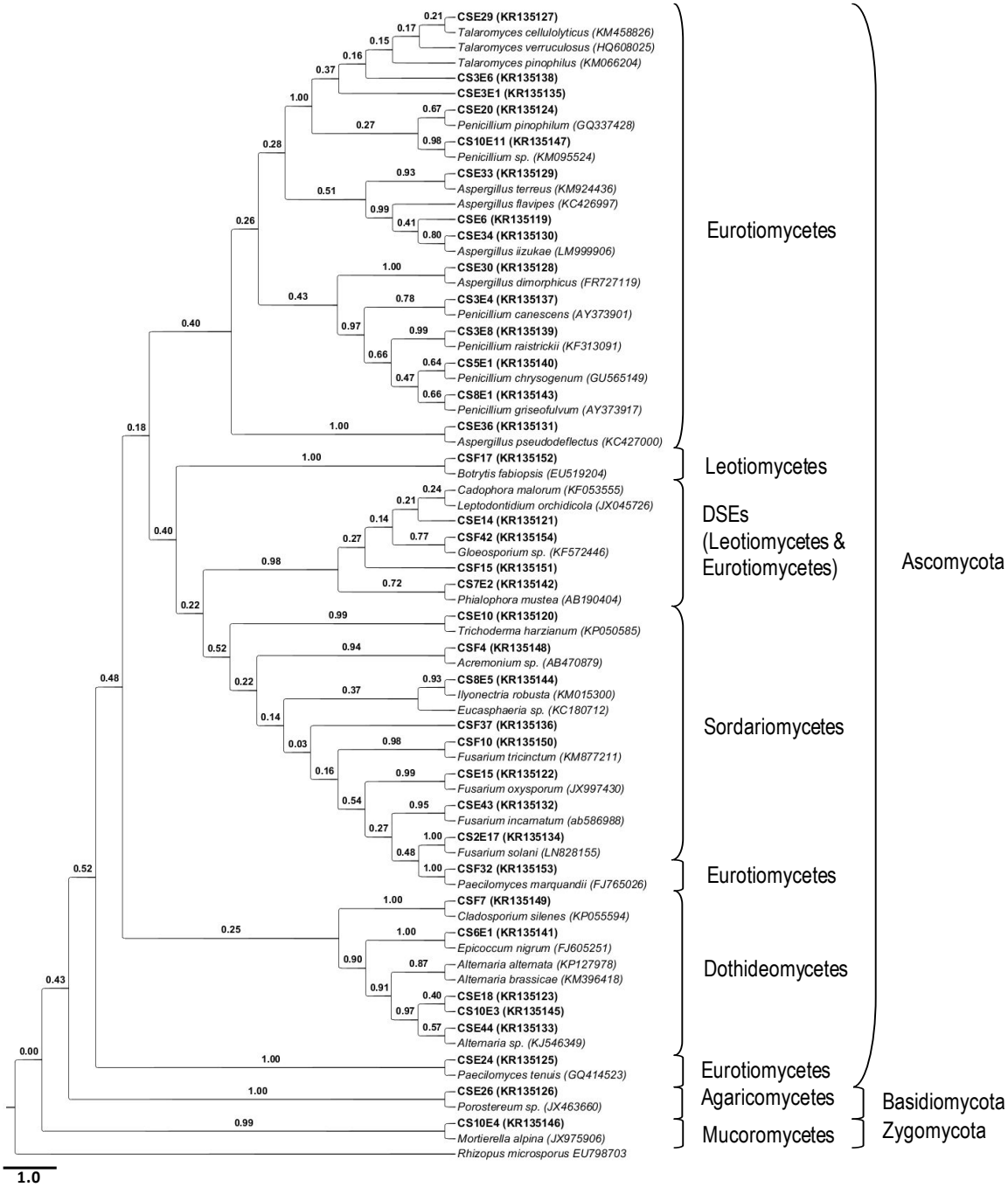
0.5
1.0
1.5
2.0
2.5
3.0
3.5
alpha

Fig. 3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Talaromyces verruculosus (HQ608025)
Talaromyces pinophilus (KM066204)
CS3E6 (KR135138)
CSE3E1 (KR135135)
CSE20 (KR135124)
Penicillium pinophilum (GQ337428)
CS10E11 (KR135147)
Penicillium sp. (KM095524)
CSE33 (KR135129)
Aspergillus terreus (KM924436)
Aspergillus flavipes (KC426997)
CSE6 (KR135119)
CSE34 (KR135130)
Aspergillus iizukae (LM999906)
CSE30 (KR135128)
Aspergillus dimorphicus (FR727119)
CS3E4 (KR135137)
Penicillium canescens (AY373901)
CS3E8 (KR135139)
Penicillium raistrickii (KF313091)
CS5E1 (KR135140)
Penicillium chrysogenum (GU565149)
CS8E1 (KR135143)
Penicillium griseofulvum (AY373917)
CSE36 (KR135131)
Aspergillus pseudodeflectus (KC427000)
CSF17 (KR135152)
Botrytis fabiopsis (EU519204)
Cadophora malorum (KF053555)
Leptodontidium orchidicola (JX045726)
CSE14 (KR135121)
CSF42 (KR135154)
Gloeosporium sp. (KF572446)
CSF15 (KR135151)
CS7E2 (KR135142)
Phialophora mustea (AB190404)
CSE10 (KR135120)
Trichoderma harzianum (KP050585)
CSF4 (KR135148)
Acremonium sp. (AB470879)
CS8E5 (KR135144)
Ilyonectria robusta (KM015300)
Eucasphaeria sp. (KC180712)
CSF37 (KR135136)
CSF10 (KR135150)
Fusarium tricinctum (KM877211)
CSE15 (KR135122)
Fusarium oxysporum (JX997430)
CSE43 (KR135132)
Fusarium incarnatum (ab586988)
CS2E17 (KR135134)
Fusarium solani (LN828155)
CSF32 (KR135153)
Paecilomyces marquandii (FJ765026)
CSF7 (KR135149)
Cladosporium silenes (KP055594)
CS6E1 (KR135141)
Epicoccum nigrum (FJ605251)
Alternaria alternata (KP127978)
Alternaria brassicae (KM396418)
CSE18 (KR135123)
CS10E3 (KR135145)
CSE44 (KR135133)
Alternaria sp. (KJ546349)
CSE24 (KR135125)
Paecilomyces tenuis (GQ414523)
CSE26 (KR135126)
Porostereum sp. (JX463660)
CS10E4 (KR135146)
Mortierella alpina (JX975906)
Rhizopus microsporus EU798703
1.0
Eurotiomycetes
Leotiomycetes
DSEs (Leotiomycetes & Eurotiomycetes)
Sordariomycetes
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Agaricomycetes
Mucoromycetes
Ascomycota
Basidiomycota
Zygomycota

Fig. 4

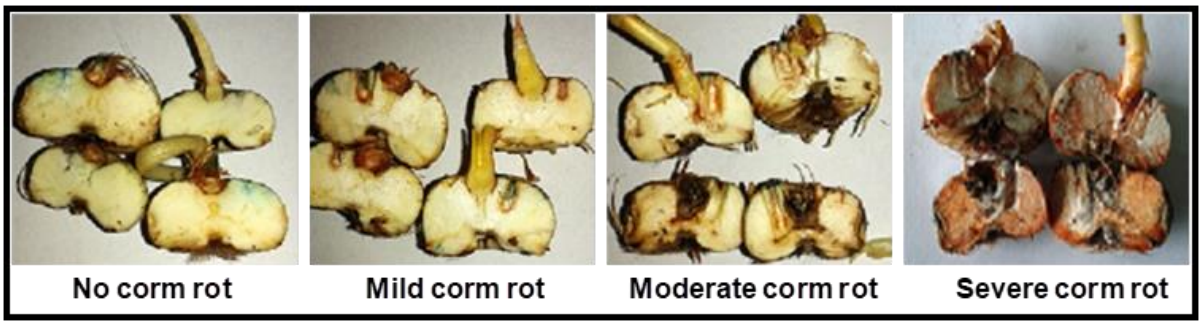

Fig. 5