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“*Crocus sativus* L. : An overview of morphological characteristics, phytochemical profile and biotechnological advances”.

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Abstract
Saffron *(Crocus sativus),* a highly priced and prominent spice of the global market has massive medicinal and industrial value, in pertinence to the pharmacological nature of the bioactive compounds present in its stigma. These bioactive constituents viz. carotenoids, flavonoids, terpenoids, apocarotenoids are the secondary metabolites (safranal, crocin, picrocrocin, crocetin) synthesized by the plant mainly for its defense and survival. These compounds also serve as deterrents against pathogens as well as herbivores, protect the plant against various environmental stresses, help in pollination, pigmentation, seed dispersal; thereby playing an active role in plant-plant interactions, plant signalling cascades and plant microbe-interactions. Their quantity of production is dependent on the prevailing environmental conditions and factors regulating the growth of corms and emergence of flowering in this plant. Moreover, the pattern of vegetative propagation of its corms hampers its genetic modification, due to which a standardised platform for its tissue culture in lab-controlled conditions becomes urgent so as to genetically improve it for the production of metabolites in gigantic amounts and further used in medicine for human welfare. This review is a concise explanation of morphology, physiology and phytochemistry of *Crocus sativus* with emphasis on its response to biotic as well as abiotic stresses and biosynthetic pathway associated with its secondary metabolite production; thus, highlighting its significance in food, cosmetics and modern medicine.

Keywords: *Crocus sativus,* safranal, crocin, picrocrocin, crocetin, abiotic and biotic stress, secondary metabolite, tissue culture.

Introduction

Crocus sativus L., generally referred as Saffron is a monocotyledonous, tuberous herb representing the flagship species within the genus *Crocus* and the family Iridaceae (Askari-Khorasgani and Pessarakli, 2019). The Iridaceae family comprises of 60 genera and 1500 species and saffron stands out as one of the most treasured and interesting species amongst 80 species of its genus. The plant is cultivated due to the presence of stigmas, which are harvested from its bright purple-coloured flowers. The presence of these stigmas has assigned it global recognition, as one of the most significant and costliest spice (Dhar *et al.,* 2017). True Saffron is a bright, crimson coloured spice with a pleasant aroma and a bitter taste and is available both in filamentous as well as powdered form. Due to its immense industrial importance on account of its use in the food, pharmaceuticals and cosmetics, it is also referred to as “Golden Condiment” or "Red Gold" (Ahmad *et al.*, 2011). As a spice, Saffron should be stored instantly after complete drying, ideally in air-tight or water-proof containers and kept in dark to prevent its bleaching. The final product is in the form of an extremely condensed, natural, entangled mass of elongated strands, dark orange to reddish brown thread-like structures about 1 inch in length (Koocheki *et al.,* 2020). Due to its high market value, this cash crop makes it prone to frequent adulterations. Saffron has huge medicinal value due to the presence of three important secondary metabolites i.e. Crocin, Picrocrocin and Safranal as its bioactive components (Cossigani *et al.,* 2014; Dass, 2017). These secondary metabolites are produced in this plant by apocarotenoid biosynthetic pathway. There are various pathway specific genes that led to the formation of these components and a lot of molecular research work like transcriptome, proteome, study of various transcription factors involved etc. is ongoing with an aim to enhance its yields for the increased production of these bioactive components. It is also being raised in tissue culture labs nowadays and its gene editing through CRISPER/CAS technology is in trails in order to manipulate its genome by overcoming male sterility and restriction of propagation on account of the vegetative method of propagation found in it.

Morphology

The *Crocus* plant consists of a corm which is a bulbous mass with tunic layers and it is sown in a good textured, loamy soil with sand and manure in proper ratio and ph around 6-8. The corm gives rise to two types of roots i.e. fibrous roots/absorbing roots and contractile roots (Choi *et al.,* 1996). The contractile roots usually goes perpendicularly downwards and they grow from base in second year corms mostly. The absorbing roots/fibrous roots grows from sides of the corms as shown in the **Fig 1**. and helps in absorbing nutrients from the soil. The corms have huge quantities of starch stored during their earlier development which leads to the initial growth of buds. The two types of buds found in *Crocus* are apical buds and lateral buds. The apical bud leads to flowering but only few or most of the time a single lateral bud leads to emergence of second flower from the same corm. The flower that emerges from the apical bud has violet-coloured petals, with yellowish stamen and a red coloured tripartite stigma (Mathew *et al.,* 1982; Fernandez *et al.,* 2006). These stigmas form the saffron of commerce, which is the novel product that makes this crop so important throughout the world.

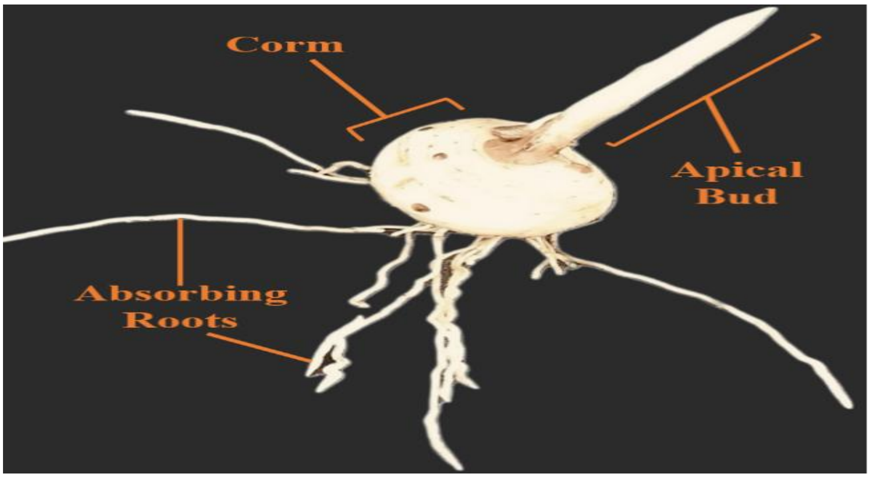

Fig 1. Corm of *Crocus* with apical bud and absorbing roots

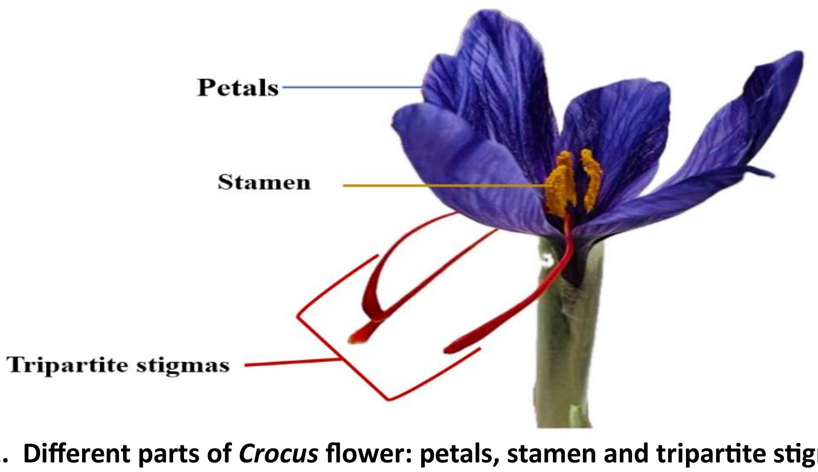

Fig 2. Different parts of *Crocus* flower: petals, stamen and tripartite stigma

History and distribution

The term Saffron comes from the French word Safran, which is evolved from the Latin word safranum and that has been derived from the Arabic word as far, meaning yellow. Saffron is native to the Mediterranean basin, probably evolved around Greece, Persia and Asia Minor (Nemati *et al.,* 2019). It is widely cultivated in the East and the Mediterranean region from the late bronze age. In Egypt and Middle East, it has been planted as a seasoning herb or a spice for not less than 3500 years. It is presently being cultivated in Iran, India, Spain, Greece, Morocco, Turkey, Azerbaijan, Italy, Israel, France, Switzerland, Pakistan, China, Japan, Egypt, United Arab Emirates and continuous efforts to introduce this condiment in non- traditional areas such as Australia, USA, Chile, New Zealand and Argentina have been made lately (Kumar *et al.,* 2008).India ranks second after Iran, which is the leading producer of saffron contributing approximately about 88% of the global production (Sharma *et al.,* 2020). There is no availability of any documentation which supports the exact time of introduction of saffron into India, especially in Kashmir, which is the only commercialized area for saffron cultivation in the country. Saffron which is being grown in Kashmir came from Persia and its cultivation expands beyond the Pampore region to alluvial plateaus of Budgam and Southern Kashmir. Nowadays, saffron is grown in Kishtwar district of Jammu as well and at various research field stations like CSIR-IHBT Palampur Himachal Pradesh (Kumar *et al.,* 2020).

Flowering in *Crocus sativus*

The productivity of *Crocus* flowers per unit area is dependent on several factors, including environmental conditions such as temperature and rainfall patterns, edaphic factors of soil, quality of planting material and farming practices (Menia *et al.,* 2018). The different parts of *Crocus* flower have been mentioned in **Fig 2**. The initiation of flowering in saffron is stimulated by the combined action of ambient temperature conditions and moisture content of the soil. Also, colder environments tend to encourage substantial flowering because of effect of low temperature on the induction of flowering, nevertheless, timing of appearance of full bloom is independent of the density of planting material (Gresta *et al.,* 2009). Climatic conditions are one of deciding factors responsible for estimating the yield of saffron, thus limiting its plantation in different areas all over the world (Bayat *et al.,* 2016) Low temperature conditions, particularly before the onset of flowering are meant to positively regulate the quantity of saffron production. Lower air temperatures are known to expedite the cessation of dormancy in the mother corms for the next growing season (Rasheed-mohassel, 2020), thereby influencing the growth of buds and appearance of root primordia at the base of the corms. (Nehvi *et al.*, 2006) Higher temperatures lead to overall reduction in the yield of saffron due to decrease in the number of flowers (Koocheki and Khajeh-Hosseini, 2020; Negbi, 1999.). Feasibility study of saffron cultivation using semi-saline water by managing its planting date has revealed that it brings delay in the process of flowering (Rashed - Mohassel, 2020). Autumn temperature conditions play a principal role in the production of stigmas, while spring is essential for the production of new corms for the upcoming year. In one study, it has been shown that the sowing depth of corm in the soil is also known to play an important role in its flowering.

Apocarotenoid Biosynthetic pathway

Carotenoids are the primary and most significant components of saffron stigmas, contributing to its distinctive features. These components include fat-soluble carotenoids like lycopene, α- and β-carotene, and zeaxanthin, as well as water-soluble C20 apocarotenoids and crocetin derivatives (Botella *et al*., 2004; Isacson *et al.,* 2004). Apocarotenoids, non-polar isoprenoids derived from the oxidative cleavage of zeaxanthin, play a crucial role in saffron, particularly in the stigma tissue (Cazzonelli *et al*., 2010). In higher plants, these compounds function as phytohormones, signaling molecules, and pigments providing color to flowers and fruits. In saffron, apocarotenoids are extensively studied due to their high concentration in stigmas and their significant cosmetic and organoleptic properties (Baba *et al.,* 2016). The biogenesis of the main crocins and safranal in saffron involves the biooxidative cleavage of zeaxanthin through a 7,8 (7',8') cleavage reaction. The quality of saffron is assessed based on the content of these bioactive compounds and their pharmacological roles. The primary components of saffron include cis- and trans-crocins, picrocrocin (a β-D-glucopyranoside of hydroxy-β-cyclocitral) and its degradation product i.e. safranal, which together contributes to saffron's color, taste, and aroma, respectively (Sharma *et al.,* 2019). These components also possess health-promoting properties and medicinal cure to various human diseases like depression, diabetes, inflammation disorders, ophthalmic diseases, cancers etc. (Gohari *et al.*, 2013).

In addition to these major compounds, various minor compounds from different classes of natural substances have been isolated from saffron stigmas, petals, and corms. Crocusatins, terpenoids present in stigma and petal, along with several monoterpene glycosidic derivatives considered precursors of volatile saffron components, have been identified. Saffron contains around 28 volatile and aroma-yielding compounds, predominantly ketones and aldehydes, with safranal being the main aroma-active compound responsible for saffron's distinctive scent. The yellow-orange color of saffron is primarily due to α-crocin, an ester form of the carotenoid crocetin, while the bitter glucoside picrocrocin contributes to its pungent flavor. Picrocrocin is a truncated version of zeaxanthin, produced via oxidative cleavage, and is the glycoside of the terpene aldehyde safranal. Flavonoids in saffron serve diverse functions, including ultraviolet protection, defense mechanisms, allelopathy, flower colouring to attract pollinators, inhibition of auxin transport, plant–microbe interactions, and regulation of reactive oxygen species. They are also essential for pollen viability in many species. Additionally, anthraquinones and anthocyanins have been reported from the corms and petals of *C. sativus*. Anthocyanins such as delphinidin, petunidin, and malvidin, which have various sugar substitutions, are responsible for the blue colour of the tepals (Dhar *et al.,* 2017).

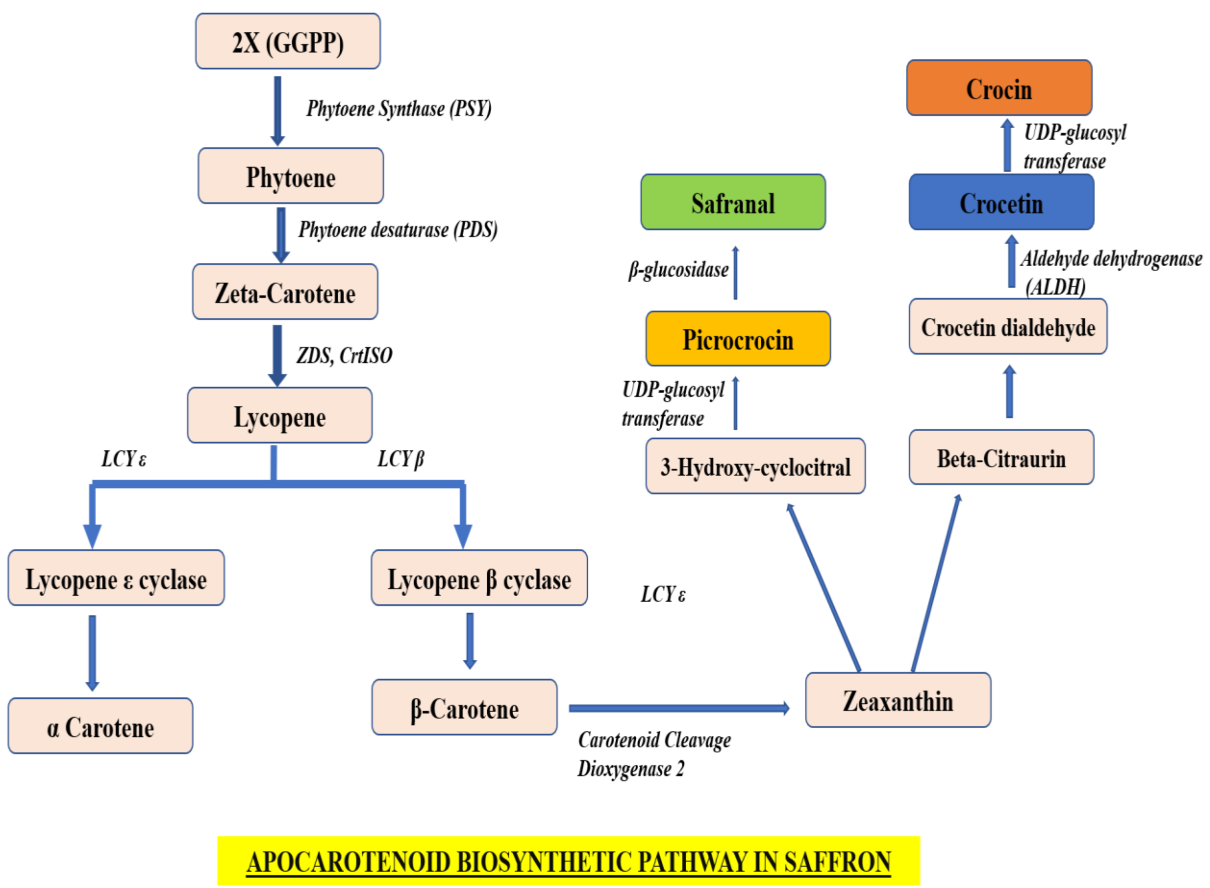

Fig 3. Pictorial representation of apocarotenoid pathway in *Crocus sativus*

Biotic stress

Crocus is susceptible to various kinds of biotic stresses. Corm rot is considered as the most threatening disease of saffron that is caused by soil-dwelling fungi like *Pythium* spp., *Rhizoctonia* spp., *Fusarium solani, Macrophomina phaseolina, Phomo crocophila* and a species of *Basidomycotina* (Bashir *et al.,* 2017; Gupta *et al.,* 2020). These diseased corms display symptoms of red, white, black and brown colour. *Macrophomina* *phaseolina* responsible for causing Charcoal rot in saffron, was originally discovered in Italy. Corm rot of saffron which is caused by *Macrophomina phaseolina* was reported in India by Thakur *et al.,* (1997) who described that 30-40% corms displayed symptoms of infection during the plantation stage of the crop. In Italy, Corm rot of *Crocus* due to *Penicillium cyclopium* was reported to happen during the period from July to August when dampness and high temperature conditions prevail. It was concluded that damaged or injured corms were more vulnerable to diseases. The initial symptoms of rotting appeared during the blooming stage, resulting into wilting and yellow-coloured shoots which was an outcome of basal stem rot and formation of white round patches on the surface of corms, followed by appearance of black powdered substance inside the outer layer of the corms. Corm rot in Saffron caused by *Fusarium oxysporum* can be controlled successfully by immersing the corm in difolatan (80% captafol), 0.2% banlate (benomyl)/ bavistin (carbendazim) each for 20 min before its germination into the soil (Gupta *et al.,* 2020; Srivastava *et al*., 2010). *Fusraium* spp. gives rise to Corm rot in the corms and white grubs attack *Crocus* crop leading to terrible losses. Implementation of Quinalphos (5 G) or Chlorpyriphos/phorate (10 G) @25-30kg/hectare is known to efficiently check the attack of white grubs (Bashir *et al.*, 2017). The crop is also liable to get infested by different kinds of viruses like bean yellow mosaic virus, arabis mosaic virus and tobacco rattle virus (Parizad *et al.,* 2016; Parizad *et al.,* 2018). Apart from fungal and viral pathogens, Saffron corms are prone to attack by mices and moles as well. Crows also bring destruction to the crop in fields, by damaging its flowers, which are the most valuable part of the plant and thus incur heavy losses. A rare pest in *Crocus* is the *Cantharidine* beetle, which in its search for honey inflicts damage to the flowers during the wee hours and thereby damages the stigmas (Feizi *et al.,* 2015).

Abiotic stress

Crocus has been able to flourish in different bio-geographical zones all over the world. It is capable of thriving in arid, semi-arid and temperate environmental conditions (Kumar *et al.,* 2009). The differences in yield and quality can be attributed to the prevailing climatic conditions and traditional agriculture practices undertaken by farmers (De Juan *et al.,* 2009). The water requirements of saffron is low and can be met by rainfall and limited irrigation. Saffron displays a poor competitive behaviour against weeds for minerals, elements, water and particularly light (Rimani *et al.,* 2019). Weeds are known to impart a negative effect on the growth of its bulbs/corms and thus development of the plant leading to significant loss in its total yield (Ramesh *et al.,* 2017). The choice of corms taken, planting density, period of planting, planting space and density, irrigation, fertilization and weed control are the factors which govern its proper growth and development.

Temperature is the most critical factor governing the growth and flowering in Saffron. It can withstand very high temperatures upto 40 ºC during summer and low temperatures upto -18 ºC during winter (Hertough and Nard, 1993) This verifies the difference in the time of appearance of flower in places with diverse climatic conditions. Photoperiod also imparts remarkable influence on the pattern of flowering. Photoperiod in the range of 10-11 hr is beneficial (Gresta *et al.,* 2009).

Drought is one of the crucial environmental factors determining the productivity of crops world-wide, subsequent to which plants tend to show curtailed growth and decrease in their overall yield (Cardone *et al.,* 2020). A decline in the growth of *Crocus* including reduced number, length and leaf area; accompanied with decrease in the size of its daughter corms was observed as a result of drought stress.

The changing climatic conditions all around the world has detrimentally affected the growth and development of plants by making them vulnerable to biotic and abiotic stresses. As a counter-effect to these stresses, plants activate their basic defence mechanism in the form of production of secondary metabolites (Sharma *et al.,* 2010), that are the key biomedical and physiological constituents. These diverse and unique compounds namely flavonoids, alkaloids, phenolics, nitrogen/sulphur containing compounds serve as natural protectants against numerous pathogens, herbivores and oxidative stress.

Tissue Culture

Saffron (*Crocus sativus* L.), is one of the most expensive crops in the world due to the presence of unique apocarotenoids available in the stigmas of the saffron, mainly safranal, crocin and picrocrocin. However, the male sterility of the crop makes it a sterile triploid (2n = 3x = 24), a genetically recalcitrant variety restricting it from any genetic improvement (Ahmed *et al.,* 2014). The only option of reproduction in the crop is

vegetative reproduction resulting into low multiplication rate and productivity. The vegetative reproduction hinders the genetic improvement and recalcitrance of saffron thus hinder efficient genetic manipulation through traditional methods (Kumar *et al.*, 2008). Plant tissue culture techniques provide a pivotal platform for *invitro* development of the crop for genetic improvement and large-scale propagation.
The initiation of saffron tissue cultures from various plant parts including leaves, shoots, corms, and flower components has been documented. Several researchers have established standardized protocols for sterilization of plant materials, including corms and flower buds. These protocols encompass various treatments to ensure the elimination of contaminants and maintenance of sterile conditions. Microbial contamination, including bacteria, fungi, yeast and viruses are the leading causes of loss in *invitro* culture. Consequently, maintaining sterile conditions is vital for the success of plant tissue cultures. Researchers aim to eliminate or suppress contamination, typically through chemical sterilization methods (Arab *et al.,* 2014). Therefore, the optimal concentration and exposure time of the sterilant is crucial to minimize explant injury and ensure successful plant tissue culture techniques (Hesami *et al.,* 2018). Several researchers have established standardized protocols for the sterilization of plant materials, including corms and flower buds. These protocols encompass various treatments to ensure the elimination of contaminants and maintenance of sterile conditions.
Saffron's vegetative propagation makes it inherently recalcitrant to genomic modifications. The facilitation of genetic transformation using tissue culture technique is a new area of research. However, the establishment of competent *invitro* systems is a prerequisite for advanced biotechnological interventions, such as Agrobacterium-mediated transformation, biolistic approaches and CRISPR/Cas-based genome editing. These strategies can potentially be exploited to modulate key metabolic pathways, particularly the apocarotenoid biosynthetic pathway, for enhanced yields and improved quality traits.

Conclusion

The present study is emphasized at evaluating the vast potential of Saffron as a product of medicinal, commercial and cultural significance. It also provides an insight into the challenges associated with the cultivation, growth and development of *Crocus,* involving various biotic and abiotic factors and effect of climatic conditions over its production. The most important and valuable part of Saffron flower are tripartite stigmas, which are a source of secondary metabolites (carotenoids/flavonoids/terpenoids/apocarotenoids) like safranal, crocin and picrocrocin. These bioactive constituents are produced by plants in defence and they also serve as pharmacological and biochemical agents. However, the vegetative mode of reproduction of this crop obstructs its faster propagation resulting into lower productivity; hence, it becomes imperative to develop tissue culture techniques for *invitro* development of this crop, so as to genetically improve it for increased yields and metabolites production as well.

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