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Genetic diversity of saffron and conservation strategies

Shahzada akhter, Devendra Kumar pandey

School of Bioengineering and Biosciences

Lovely professional university, Jalandhar, Punjab

Abstract

Crocus sativus L. (common name: Saffron; family: Iridaceae) is a very important spermatophyte used extensively in spice and coloring similarly as a flavorer agent. Among eighty five species reportable beneath the liliid monocot genus, Saffron (C. sativus) is that the most fascinating and commercially necessary species. Saffron could be a polyploid (3n) and sterile herb propagated naturally by corms, originated from mother corms. within the ancient system of drugs, it's been reportable wide for its profound therapeutic efficacy; viz. anti-depressant, aphrodisiac, analgesic and expectorants. Saffron stigma is that the potent supply of bioactive compounds and a vital marketed spice similarly as a food colorant. The valuable spice, Saffron and its bioactive apocarotenoids area unit acknowledged for his or her therapeutic effects on neurodegenerative diseases exhibiting anti-convulsant, anti-depressant, anti-anxiety, anti-ischemia, anti-Parkinsons', anti-Alzheimer's and anti-nociceptive activities. The principle pigments of saffron area unit crocin, safranal, organic compound picrocrocin and crocetin. However, there has been very little success in enhancing the degree of those bioactive apocarotenoids of economic importance. The genetic study has shown that this plant represents varicolored ploidy pattern and also the prevalence of genetically heterogenous forms—indicating the chance of organism choice, mutation breeding and condition induction within the development of recent high yielding cultivars of C. sativus. Tissue culture is another, property and biotechnologically advance technique for giant scale production of infection free stalk biomass of iridaceous plant. This review illustrates therapeutic potential of saffron and its active phytoconstituents, their extraction technique, analysis of genetic diversity, internal control assessment and property production of bioactive compounds harvested from C. sativus.

Keywords: saffron, Spermatophtyte, monocot, polyploid, propagated, analgesic, Tissue culture, extraction.

Introduction

Saffron (Crocus L.) is an individual from Crocoideae, the greatest of four subfamilies in the Iridaceae family.It has 2n = 3x = 24 chromosomes and is triploid; accordingly, it is sterile. In past research, distinctive sub□atomic DNA markers were utilized yet sub-atomic portrayal and hereditary assorted variety of this mind boggling family have not yet been explained. Along these lines, current investigation expected to decide the atomic portrayal of saffron and its nearby relative species utilizing between groundwork restricting site (iPBS)- retrotransposon markers. Eighty-three iPBS-retrotransposon groundworks were utilized in 28 C. sativus genotypes and 17 close relative types of saffron to recognize their hereditary assorted variety. Sixteen polymorphic iPBS-retrotransposon groundworks created an aggregate of 401 polymorphic scorable groups. The mean PIC esteem, Nei's hereditary assorted variety and Shannon's data file (I) were determined as 0.85, 0.16 and 0.29, individually. The aftereffects of the Unweighted Pair Group Method with Arithmetic mean UPGMA dendrogram and Principal Coordinates Analysis PCoA examination showed a spatial portrayal of the relative hereditary separations among 28 saffron tests and the 17 close relative species were ordered under two particular gatherings. Saffron genotypes demonstrated constrained hereditary variety and as indicated by the iPBS-retrotransposon information, its nearby family members were C. cartwrightianus and C. pallasii subsp. Pallasii. Saffron (Crocus sativus L.), one of the most costly flavors on the planet, is utilized predominantly as nourishment shading and enhancing in nourishment industry and its successful parts are additionally utilized in medication. An assortment of twenty-two cultivars of saffron developed in various areas of Iran was screened with 25 SSR and 5 SNP preliminaries so as to decide hereditary personalities and hereditary decent variety in these cultivars.

Saffron got GI Tag because saffron is famous in kashmir. Documentation Number of saffron is 635 . Within J&K, there are four districts which are climatically more favourable for saffron cultivaton i, e Pulwama, Budgam, Kishtwar and Srinagar. The production area of each district varies from one another, pulwama contributes 3200 ha of land, srinagar 165 ha and Budgam 300ha and kishtwar 120 ha. There are three grade of saffron that are available in kashmir_ Lacha saffron, Mongra saffron and Zarda saffron. krewa soil is suitable for saffron. Saffron is a Rabi crop. The stigma and sometimes the petals are also used to make medicine. The demand for saffron is increasing worldwide for it's interesting role in cuisine, medicine and cosmetics. It is largely cultivated in Iran, India, Afghanistan, Greece, Morocco, Spain, and Italy. Iran is the largest producer of saffron in the world. Among the world's total saffron production of 205 tons, Iran contributes 160tons (80%), J&K contributes around 8-10 tons (~5%)

Tissue culture

Tissue culture (TC) is the cultivation of plant cells, tissues, or organs on specially formulatednutrient media. Under the right conditions, an entire plant can be regenerated from a single cell.Plant tissue culture is a technique that has been around for more than 30 years. Tissue culture is seen as an important technology for developing countries for the production of disease-free, high quality planting material and the rapid production of many uniform plants.

Table1: Tissue culture table

Species	Plant part used in tissue culture	Ms media and pgr	Results	References
Crocus sativus	dried stigmas		The highest yields were obtained in the second year by the combination of 'Genzano di L. × Sardinia' and 'Castelgrande × Abruzzo' with 28.1 and 23.9 kg ha−1 of dried stigma, respectively.	Cardone, L., Castronuovo, D., Perniola, et al
Crocus sativus	Explant	(0.5, 1, 2 and 4 mg L-1 2,4-D or NAA in combination with 0.5 and 1 mg L-1 Kin or BAP Ms media	sonication of the saffron corm explants significantly increased the in vitro callus induction and growth. So, the highest callus induction (100%) and yield (4.68 g) was achieved with sonicated explants cultured on MS medium supplemented with 2 mg L-1 1-naphthaleneacetic acid (NAA) and 0.5 mg L-1 kinetin (Kin).	Firoozi, B., Zare, N., Sofalian, et al
Crocus sativus	corm and style explants of saffron	MS medium with name thidiazuron (TDZ), benzylaminopurine (BA), 1-naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D)	In general, style-derived calli showed longer time survival with a fine texture and good quality compared to corm-derived calli.	Moradi, A., Zarinkamar Et al
Crocus	saffron	MS media With BAP and	The in vitro	Soukrat, S.,

sativus	corms	NAA	micro-propagation seems to be a very promising technology for saffron production and will help in the diffusion of any genetic progress generated by the current participatory breeding program of saffron in Morocco.	Benlhabib Et al
Crocus sativus	callus induction and regeneration of saffron using thin cell layer explants	MS medium supplemented with different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D), 6-BenzylAminoPurine (BAP) and 1-NaphthaleneAcetic Acid (NAA)	The results of this investigation revealed that the thin cell layers from buds are suitable explants for regeneration	Azadi, P., Bagheri et al
Crocus sativus	corm explants	(MS) Medium or MS with several dose of BAP	result in foliation from the initiated shoots in 90 days	Cavusoglu, A., Sulusoglu et al
Crocus sativus	roots and corms.	Murashige and Skoog (MS) media with 3% (w/v) sucrose, 100 mg L-1 ascorbic acid, and the combination of 0. 25 mg L-1 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg L-1 6-benzylaminopurine (BAP) and 1 mg L-1 indole-3-butyric acid (IBA) was more preferable for adventitious corm and root initiation as well as growth	Overall, 36% and 57% of explants had corm and contractile root, respectively.	Zeybek, E., Önde, Et al
Crocus sativus	Dried stigmas	MS medium 2,4-D and BA	Basal parts of leaves were used as explants. Green callus was induced in 2.5 ppm 2,4-D and 0.3 ppm BA.	Sharafzadeh, S. Et al

Crocus sativus	ovary and style explants of floral buds	MS medium supplemented with 1-naphthalene acetic acid (NAA) and 6-benzlaminopurine (BAP)	Our results suggest that the SLS originated from internal parenchyma tissues.	Namin, M.H., Ebrahimzadeh, Et al
Crocus sativus	shoots	Murashige and Skoog (MS) medium containing 3 mg dm-3 benzyladenine (BA)	Daughter cormlet formation from in vitro derived cormlets was also observed.	Sharma, K.D., Rathour et al
Crocus sativus	pathogen-free corms.	MS medium without growth regulators	microcorms were produced at the basal part after 3 months.	Sheibani, M., Nemati, Et al
Crocus sativus	leaf segments of saffron	MS medium containing BA (4.0 mg L-1) + NAA (0.50 mg L-1) + 9% sucrose	An average of 10.82 shoots per explant of proliferated culture was obtained when MS medium was supplemented with BAP (2.0 mg L-1) + NAA (0.50 mg L-1).	Raja, W., Zaffer et al
Crocus sativus	ovary explants	Murashige and Skoog (1962) medium was supplemented with naphthaleneacetic acid and benzyladenine	Further development of ovary-derived shoots was influenced by the composition of basal salts in the culture medium	Bhagyalakshmi, N. Et al
Crocus sativus	Callus	Ms media and auxin, cytokinin and coconut milk	An increase in 2,4-D level enhanced callus formation but suppressed shoot bud formation.	Ilahi, I., Jabeen, Et al
Crocus sativus	bud explants	Cytokinins and auxin and ethylene.	Microsurgery of the apical bud combined with ethylene pretreatment increased both sprouting and corm production.	Plessner, O., Ziv et al,

Crocus sativus	floral and corm segments	nutrient media supplemented with various concentrations of plant growth regulators	Regenerated corms were kept at 5°C for 5 weeks and then transplanted to a potting mixture.	Karaoğlu, C., Çöcü, et al
Crocus sativus	explants	nutrient media supplemented with various concentrations of plant growth regulators	SLS were obtained in G-5 medium	Mir, J.I., Ahmed, et al

Selection of plant

Some scientists use stigma of saffron for extraction of croccin.

Sterilization

Sterilization is the complete removal of microorganisms from an object or surfaces.

Sterilization is obtained when microorganisms are subjected to antimicrobial agents for sufficient time and at optimum conditions. Some physical methods associated with sterilization are explained below:

Heat Sterilization

Heat sterilization is the most effective and widely used method of sterilization, where the bactericidal activity results through the destruction of enzymes and other essential cell constituents.

The effects of heat sterilization occur more rapidly in a fully hydrated state, as it requires a lower heat input, with low temperature and less time, under high humidity conditions where the denaturation and hydrolysis reactions are predominant, rather than in the dry state where oxidative changes take place.

This method of sterilization is applicable to thermostable products. Still, it can be applied to both moisture-sensitive and moisture-resistant products, for which dry (160–180°C) and moist (121–134°C) heat sterilization procedures are respectively used.

Moist Heat Sterilization

Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.

Moist heat sterilization usually involves the use of steam at temperatures in the range 121–134°C.

High pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.

The most commonly used standard temperature-time cycles for clinical porous specimens (e.g. surgical dressings) and bottled fluids are 134°C for 3 minutes and 121°C for 15 minutes, respectively.

An autoclave is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.

In this method, the microorganisms are killed by coagulating their proteins, and this method is much more effective than dry heat sterilization where microbes are killed through oxidation.

In the pharmaceutical and medical sectors, it is used in the sterilization of dressings, sheets, surgical and diagnostic equipment, containers, and aqueous injections, ophthalmic preparations, and irrigation fluids, in addition to the processing of soiled and contaminated items.

Moist heat can be used in sterilization at different temperatures:

At temperatures below 100°C

The sterilization technique employed at a temperature below 100°C involves pasteurization.

In this process, all non-spore forming microbes are killed in milk by subjecting the milk to a temperature of 63°C for 30 minutes (the holder method) or 73°C for 20 seconds (the flash method).

In pasteurization, however, not all the pathogenic organisms are killed. The principle of pasteurization is the logarithmic reduction in the number of viable microbes so that they can nolonger cause diseases. The milk is not heated above its boiling point as the milk might curdle, and its nutritional value might be destroyed.

Besides milk, other fluids and equipment like vaccines of non-sporing bacteria are also pasteurized at 60°C for 1 hour in special water baths.

Similarly, serum and body fluids with congealable proteins are also sterilized at 56°C for 1 hour in water baths.

At a temperature of 100°C

Boiling at 100°C is a moist heat sterilization technique that doesn't ensure complete sterility, but is enough for the removal of pathogenic vegetative microbes and some spores.

In this case, the items to be sterilized are immersed in boiling distilled water for 30-40 minutes.

Distilled water is preferred because hard water might result in the formation of a film of calcium salts on the instruments.

Tyndallization is a method that is used for sterilization of media with sugar and gelatin at 100°C for 30 minutes on three successive days so as to preserve sugar which might be decomposed at a higher temperature.

Moist heat at 100°C is applicable for contaminated dishes, beddings, pipettes, and other instruments that are not soiled or contaminated as well as for objects that are temperature sensitive.

At temperatures above 100°C

Moist heat sterilization above 100°C involves sterilization by steam under pressure.

Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.

This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.

As a result, the steam under pressure has a higher penetrating power. When this steam comes in contact on the surface, it kills the microbes by giving off latent heat.

The condensed liquid ensures the moist killing of the microbes.

Autoclaves are used for the sterilization of contaminated instruments along with different culture media as it ensures complete sterility.

Dry heat sterilization

Dry sterilization is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.

The dry heat sterilization process is based on the principle of conduction; that is the heat is absorbed by the outer surface of an item and then passed onward to the next layer. Ultimately, the entire item reaches the proper temperature needed to achieve sterilization.

Dry moisture-less heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration

Dry heat sterilization is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons. Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.

Thus, in dry heat sterilization usually higher temperatures in the range 160–180°C are employed and also require exposure times of up to 2 hours depending upon the temperature employed.

This principle is used in instruments like hot air oven and incineration, which generates very hot moisture-free air.

There are different types of dry heat sterilization which are explained below:

Red Heat

Rest heat sterilization is the process of instant sterilization by holding the instruments in a Bunsen flame till they become red hot.

This method is based on dry heat sterilization is commonly used for sterilization of instruments like incubation loops, wires, and points of forceps.

This process ensures effective sterilization; however, it is only limited to substances that can endure heating until redness in flame.

Flaming

Flaming is a type of dry sterilization that involves exposure of metallic objects to flame for some time where the flame burns microbes and other dust presents in the instrument.

In the case of flaming, the instrument is dipped in alcohol or spirit before burning it in a gas flame. This process doesn't ensure sterility and is not as effective as red hot sterilization.

Incineration

Incineration is the process of sterilization along with a significant reduction in the volume of the wastes. It is usually conducted during the final disposal of the hospital or other residues.

The scraps are heated till they become ash which is then disposed of later. This process is conducted in a device called incinerator.

Infrared radiation

Infrared radiation (IR) is a method of thermal sterilization in which the radiation is absorbed and then converted into heat energy.

For this purpose, a tunnel containing an IR source is used. The instruments and glassware to be sterilized are kept in a tray are then passed through the tunnel on a conveyer belt, moving at a controlled speed.

During this movement, the instruments will be exposed to the radiation, which will result in a temperature of about 180°C for about 17 minutes.

IR is applicable for mass sterilization of packaged items like syringes and catheters.

Hot air oven

Hot air oven is a method of dry heat sterilization which allows the sterilization of objects that cannot be sterilized by moist heat.

It uses the principle of conduction in which the heat is first absorbed by the outer surface and is then passed into the inner layer.

A hot air oven consists of an insulated chamber that contains a fan, thermocouples, temperature sensor, shelves and door locking controls.

The commonly-used temperatures and time that hot air ovens need to sterilize materials are 170°C for 30 minutes, 160°C for 60 minutes, and 150°C for 150 minutes.

These ovens have applications in the sterilization of glassware, Petri plates, and even powder samples.

Filtration

The process of filtration is unique among sterilization techniques in that it removes, rather than destroys, microorganisms.

Further, it is capable of preventing the passage of both viable and nonviable particles and can thus be used for both the clarification and sterilization of liquids and gases.

The primary mechanisms involved in filtration are sieving, adsorption, and trapping within the matrix of the filter material.

Filtration uses membranous filters that have tiny pores that let the liquid pass through but prevent bigger particles such as bacteria from passing through the filter. Therefore, the smaller the pore, the more likely the filter is to stop more things from going through it.

The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections and ophthalmic solutions, biological products, air, and other gases for supply to aseptic areas.

They may also be required in industrial applications where they become part of venting systems on fermenters, centrifuges, autoclaves, and freeze dryers.

Filtration sterilization of liquids

Membrane filters, in the form of discs, can be assembled into pressure-operated filter holders for syringe mounting and in-line use or vacuum filtration tower devices for filtration of liquid.

Filtration under pressure is generally considered most suitable, as filling at high flow rates directly into the final containers is possible without problems of foaming, solvent evaporation, or air leaks.

Filtration sterilization of gases

Filters employed for this generally consist of pleated sheets of glass microfibres separated and supported by corrugated sheets of Kraft paper or aluminum which are employed in ducts, wall or ceiling panels, or laminar air flow cabinets.

These high-efficiency particulate air (HEPA) filters can remove up to 99.997% of particles >0.3mm in diameter and thus are acting as depth filters.

In practice, their microorganism removal efficiency is rather better as the majority of bacteria are found associated with dust particles.

What is an Autoclave?

The invention of the autoclave sterilizer is attributed to Charles Chamberland, in 1879. Around that time, researchers started to understand the advantages of sterile surgery, and doctors needed a more reliable sterilization method than open flaming. The autoclave's benefits were soon evident, and it became an essential part of every clinic and hospital.

An autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes.

Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

What is an Autoclave Used for?

An autoclave chamber sterilizes medical or laboratory instruments by heating them above boiling point.

Most clinics have tabletop autoclaves, similar in size to microwave ovens. Hospitals use large autoclaves, also called horizontal autoclaves. They're usually located in the the Central Sterile Services Department CSSD) and can process numerous surgical instruments in a single sterilization cycle, meeting the ongoing demand for sterile equipment in operating rooms and emergency wards.

Organogenesis

The development of plant organs is initiated from the meristematic cells. The organs above the root, like lateral organs, are initiated by the SAM (shoot apical meristem). The leaves, which regenerate from SAM,

maintain the organogenic capacity in their margins. These cells, when induced in-vitro, give rise to whole plants. The process is known as organogenesis.

Organogenesis is defined as the development of organs, like roots, shoots, and flowers, either directly from an explant, or from the callus culture. The embryo is not considered an organ because of the absence of a vascular system and its independent existence.

There are three ways of organogenesis (by which adventitious organs form): (1) from the callus culture, (2) from an explant, and (3) from the axillary bud. The organogenesis by axillary bud development can be used to regenerate the whole plant from some types of tissue culture

The process of organogenesis involves two steps: dedifferentiation and redifferentiation.

Dedifferentiation results in the formation of callus from the explant tissue with accelerated cell division.

Whereas, redifferentiation causes the development of primordia from a group of callus cells.

The process of organogenesis is affected by three factors: (a) the inoculum, (b) the medium, and (c) the environmental conditions. So, the process can be regulated by the components of media, the substances carried by the explant, and the endogenous compounds produced in culture.

CHEMICAL REGULATION OF ORGANOGENESIS

Auxin

Skoog (1944) showed that chemical supplements in media can regulate the process of organogenesis. In his experiments, he observed that the addition of auxin to the media stimulates formation of the root while inhibiting formation of the shoot; and formation of the shoot is induced by increasing the concentration of the sucrose and inorganic phosphate in the media. By looking at the observation he and his colleagues obtained, he concluded that the ratio of auxin and cytokinin controls the process of organogenesis.

The formation of the root is observed at the higher concentration of auxin. The other factors that control the formation of the root in artichoke include: mineral salt, sugar, temperature, and light. It has also been observed that the addition of exogenous auxin in culture media inhibit the root formation.

And later, many research studies showed that the endogenous auxin, cytokinin, is the determining factor of root initiation.

Cytokinin

The process of shoot initiation is known as caulogenesis. It is induced by the optimal concentration of cytokinin in the media. In labs, a suitable balance of exogenous auxin : cytokinin ratio gives rise to the formation of shoots. When any of these growth regulators are omitted, it leads to the development of buds.

The initiation of callus development occurs when the auxin : cytokinin ratio is in the range of 10 to 100. The transfer of culture from callus inducing medium to shoot inducing medium is called “reversal transfer”.

In some monocot plants, higher cytokinin to auxin ratio is required to induce the shoot initiation.

However, in some plants, only the omission of the auxin from the media results in the shoot development.

Some other compounds that can act as a substitute for the cytokinin in the culture media include purines, pyrimidines, and urea. The addition of adenine sulfate is very efficient in the shoot development or the induction of buds.

WHAT ARE THE ENDOGENOUS FACTORS THAT MAY AFFECT ORGANOGENESIS?

Gibberellins: These hormones suppress both the root and shoot formation. They lower the starch content in bud-forming cells, which is required for the bud initiation.

Ethylene: This hormone blocks the organogenesis but enhances the development during primordia formation. However, in some cultures, like tobacco cotyledon and Lilium bulb tissue, ethylene is found to induce the bud initiation.

Carbohydrates: They function as a respiratory energy source and osmotic agent. The osmoregulatory function can be maintained by partial replacement of the sucrose with mannitol. The osmotic stress in cultures affects callus growth and morphology.

Organogenesis is a powerful technique to understand the mechanism of the action of hormones and other plant growth regulators, cell and tissue-type differentiation, initiation of the development of plant organs, and other molecular mechanisms of the plant.

Different techniques for extraction of saffron

Saffron is an improved pool of bioactives counting crocins, crocetin, safranal, picrocrocins, basic oils, minerals and follow sums of B1 and B2 vitamins. Getting any important fixings like bioactive compounds which are actually show in plants is totally depending on the extraction and refinement strategies.

Extraction methods of saffron bioactives

Extraction of bioactive compounds from distinctive plant compartments is exceptionally vital in their encourage executions in health-promoting nourishment and nutraceutical items. It is announced that appropriate extraction course can collect the target bioactives up to 5 times, compare to the conventional strategies. Extraction methods from conventional to the progressed ones ought to be optimized concurring to the sort of bioactive compounds accessible within the explored plant and natural qualities of the plant itself, e.g. tissue complexity, warm sensibility, etc. (El Asbahani et al., 2015). Thus, considering the assortment of plants and the significant tissues, followed by selecting the most excellent extraction strategy can ensure the effective extraction of bioactives from plant materials.

Conventional methods for extraction of saffron bioactives

Soxhlet extraction

Soxhlet extraction strategy which is respected as the reference strategy for extraction of fats, oils, basic oils and bioactive compounds was created for the primary time by German chemist Soxhlet in 1879. To do this, a small portion of dry example is put into a thimble.The thimble is at that point set in distillation flask which contains the specified solvent(s). Within the wake of coming to an flood level, the thimble holder containing solvent-solute blend is suctioned by a siphon. Siphon purges the arrangement once more into the refining flask. This dissolvable passes on isolated solutes into the bulk dissolvable. Solute is remained within the refining store and the dissolvable goes back into the plant tissue lattice. The method runs over and over until the point when the extraction is wrapped up (Azmir et al., 2013).The natural or aqueous-based dissolvable extraction of saffron bioactive compounds by means of Soxhlet extractor was detailed by Corti, Mazzei, Ferri, Franchi, and Dreassi (1996), Feizzadeh et al. (2008),), Parizadeh, Ghafoori Gharib, Abbaspour, Tavakol Afshar, and Ghayour-Mobarhan (2011) and Samarghandian, Borji, Farahmand, Afshari, and Davoodi (2013) mainly to investigate therapeutic and anti-cancer attributes of saffron compartments. The main drawbacks of Soxhlet extractor compared to novel or complementary extraction methods are prolonged extraction times, high solvent and energy consumption, impurity of the extract, low safety, and low extraction efficiency (Gupta, Naraniwal, & Kothari, 2012). So, modification of soxhlet procedure by combination with advanced extraction routes can boost its application for extraction of bioactive compounds from plant materials.

Solvent extraction or maceration/soaking

The maceration approach was utilized in hand made arranging of tonic from very a whereas. It turned into a well-known and sensible approach to urge essential bioactive fixings and basic oils. In arrange to extricate at research facility and pilot plant scale, maceration is basically comprised of a couple of stages. At first, smashing of dried plant materials into small pieces is done to construct an amplified surface zone to uncover plant compartment onto the dissolvable. Moreover, in maceration prepare, a dissolvable named menstruum is poured into a closed vessel. At last, the fluid is pushed off; be that as it may, the marc which is the strong portion of extraction strategy is crushed to recoup broad levels of hindered arrangements. The collected strained segment and the liquid come about from press are mixed and filtrated to overlook debasements (Azmir et al., 2013).In numerous cases, the target plant tissues are uncovered to the heat-acid medications or a few chemical alterations (El Asbahani et al., 2015).An broad run of solvents counting water, natural solvents and their combination have been locked in to extricate saffron bioactive compounds (Sarfarazi et al., 2015). For case, water-soluble carotenoids (i.e. crocin and its subordinates) which are liberally found in saffron can be successfully isolated from disgrace misusing the watery or aqueous-based extraction procedures. Water, methanol, ethanol and petroleum ether are habitually utilized solvents to extricate saffron bioactive compounds (Kakouri et al., 2017; Rahaiee et al., 2015). The ethanol water dissolvable at diverse proportions (e.g. 50:50 and 70:30) was found to be the leading media to extricate polyphenols and other bioactive compounds from saffron plant (Ferrara, Naviglio, & Gallo, 2014; Masi et al., 2016; Sánchez-Vioque et al., 2016). It ought to be pointed out that, the bioactives obtained by natural solvents may contain follow sums of buildups and sullies, made them unsafe for pharmaceutical and nutraceutical application

Hydro or steam distillation

Bioactives and fundamental oils can moreover be recuperated from plant tissues by hydro/steam-distillation method. It bargains with water, steam or the combination uncovered utilize of natural solvents and it ought to be performed some time recently drying of plant materials. In hydrodistillation, the plant materials are stuffed in a still compartment; the satisfactory sums of water are included in carafe and a while later warmed up to bubbling point. On the other hand, hot water and steam are respected as the elemental powerful implies to free bioactive compounds from plant measurements. It ought to be considered that tall extraction temperature makes a few eccentric misplaced in bioactive substance. This impediment limits its broad utilize in extraction of thermo-sensible bioactive compounds (Azmir et al., 2013).

Novel methods for extraction of saffron bioactives

Emulsion liquid membrane (ELM)

ELMs are outlined for extricating metal particles from dangerous effluents, wastewater treatment and recuperation of biotechnological and bioactive compounds. Really, ELM frameworks (Fig. 2) are twofold emulsions (W1/O/W2) in which W1 is the inner fluid stage containing stripping operators, O is natural film stage containing diluent, surfactant, carrier and in a few cases co-surfactant, and W2 is the outside watery stage (nourish stage) enhanced with the target fixings e.g. metal particles, phenolic compounds, natural acids, etc.

Recently, Mokhtari and Pourabdollah (2013) created an ELM framework to extricate saffron bioactive compounds from watery arrangement of dried saffron marks of disgrace. The extraction optimization by one factor-at-a time strategy shown the taking after conditions: Span 80 concentration of 2.5% as surfactant, n-decane as diluent in film stage, stage proportion (inside fluid stage to layer stage) of 0.8, treat proportion (emulsion to outside watery stage) of 0.3, and disturbing speed of 300 rpm. They detailed

that beneath the optimized conditions, more than 90% of saffron bioactives (i.e. safranal, picrocrocin and crocins) were collected into the watery stage of emulsion globules (Mokhtari and Pourabdollah, 2013).

Microwave-assisted extraction (MAE)

The dispersal of electromagnetic waves at a recurrence extend of 300 MHz to 300 GHz within the enlightened border can initiate microwave warming. In any case, the habitually utilized frequencies in magnetrons (i.e. microwave broilers utilized in household and research facility) for nourishment, therapeutic, pharmaceutical, and nutraceutical implies are 0.915 GHz and 2.45 GHz (Hao, Han, Xue, & Deng, 2002). Restricted to classical dissolvable extractions in which the mass and warm exchange happens fromthe interior to the exterior, and bad habit versa, individually, the both warm and mass exchange of MAE happens in a same course from interior of plant fabric to the dissolvable medium. One pot heat-mass exchange marvel comes about in an quickened solute exchange, collecting more bioactive compounds in amazingly brief times. Depending on the connected microwave control and characteristics of plant fabric (water substance, surface complexity and its misfortune figure), the warm exchange and temperature rise will be diverse (Bricklayer, Chemat, & Vinatoru, 2011). The extraction of bioactive compounds by conventional methods may introduce many unwelcome components to the extracted solutes which affect its quality, purity and appearance. In contrast, novel extraction practices like MAE underrate the presence of undesired by-products, save time and energy, and cause minimum solvent consumption (Chemat, Abert-Vian, & Fernandez, 2012).

Ultrasound-assisted extraction (UAE)

The extraction of bioactive compounds utilizing UAE has been created for recuperation of smell compounds, colors, micronutrients, and antioxidant specialists from different creature and plant sources (Chemat et al., 2017). As depicted in Table 4, the UAE of crocetin esters, crocin subsidiaries, saffron unstable compounds (safranal and isophorone), picrocrocin and bioactives like kaempherol, quercetin and myricetin from saffron plant has been explored. From Table 4, different watery and natural solvents or their combination can be utilized at the recurrence extend of 20–60 kHz to extract saffron bioactive compounds. The foremost noticeable properties of UAE for saffron bioactives are higher abdicate, brief extraction time and working at surrounding temperatures. Verma and Middha (2010) concluded that due to no extreme warm era duringthe operation of UAE, no noteworthy modification of bioactive compounds happened. Maggi et al. (2009) explored the UAE of saffron unstable compounds from various commercial saffron assortments with diethyl ether and cyclohexane as solvents and energetic headspace desorption.Their discoveries uncovered that UAE can deliver a more prominent degree of unstable fixings, whereas energetic headspace strategy was speedier and required less sum of saffron tests to follow the saffron unstable model.

High hydrostatic pressure (HHP) extraction

Shinwari and Rao (2018) considered the HPP extraction of saffron bioactive compounds. The connected weight run was 1000–6000 bars in affiliation with climbed extraction temperatures of 30–70 °C. The HPLC examination illustrated that increment within the applied weight caused a exceptional increment in crocin, picrocrocin, and safranal substance by adjacent 52–63%, 54–85%, and 55–62%, respectively.Though, the hoisted temperatures caused a impressive drop(25–36%) in crocin substance. The operation parameters of 5800 bar at 50 °C for 5 min were respected as the ideal parameters for HHP extraction of saffron

bioactive compounds. The HHP extracated bioactives were more effective (28%) in extinguishing cancer cells compared to the bioactives collected by classical strategies (Shinwari & Rao, 2018).

Supercritical fluid extraction (SFE)

SFE is an extraction technique which utilizes supercritical liquids as extraction solvents. The common supercritical dissolvable for SFE is carbon dioxide (CO2) which is known as a green dissolvable without any harmfulness or side impacts. Its comparative thickness as fluids, moo thickness and tall diffusivity has turned CO2 as a specific liquid in SFE. In common, less polar fragments such as safranal in saffron can be isolated by CO2-mediated SFE. Fluid or natural solvents (e.g. ethanol) are utilized at first to evacuate polar sections (crocin, crocetin, etc.) from saffron(Goleroudbary & Ghoreishi, 2016).

Table2: This table shows the extraction and analysis of croccin.

Plant part/plants	Extraction method	Solvents used for extraction	Title of the paper	Refrences
Stigma	HPLC analysis	ethanol-water or acetonitrile	Development of an improved procedure for extraction and quantitation of safranal in stigmas of Crocus sativus L. using high performance liquid chromatography	Loskutov, A.V., Beninger, C.W., Hosfield, G.L., Sink, K.C.et al
Stigmata of Crocus sativus	thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).	crocetin-glycosyl esters	Comparative analysis of crocetin and its glycosyl esters from Crocus sativus L. and Crocus haussknechtii Boiss. as an alternative source of saffron	Radjabian, T., Saboora, A., Naderimanesh, H., Ebrahimzadeh, H.et al
Saffron spice	UV-vis and HPLC	crocetin esters, picrocrocin, and two kaempferol	Effect of centrifugal ultrafiltration on the composition of aqueous	Sánchez, A.M., Carmona, M., Prodanov, M., Alonso, G.L.

		glycosides	extracts of saffron spice (Crocus sativus L.)	Et al
saffron stigmas	liquid chromatographic tandem mass spectrometric validated	Pinnacle II Cyano (5 µm 150 × 2.1 mm) column and acetonitrile : water (70:30, v/v)	Analysis of saffron (Crocus sativus L. Stigma) components by LC-MS-MS	Verma, R.S., Middha, D.et al
saffron corms	gas chromatography-mass spectrometry (GC-MS analysis	N-methyl-N-trimethylsilyl trifluoro acetamide (MSTFA) + 1% trimethyl ido silane (TMIS).	The study on phenolic compounds in Iranian Crocus sativus L. corms by GC-MS analysis (2010) Acta Horticulturae, 850, pp. 175-178.	Esmaeili, N., Ebrahimzadeh, H., Niknam, V., Mirmasoumi, M., Safarian, S., Abdi, K. Et al
saffron samples	ultrasound assisted extraction-gas chromatography-mass spectrometry and ultrasound assisted extraction-gas chromatography-olfactometry.	4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde and 3,5,5-trimethyl-2-cyclohexene-1-one.	Changes in saffron volatile profile according to its storage time (2010) Food Research International, 43 (5), pp. 1329-1334.	Maggi, L., Carmona, M., Zalacain, A., Kanakis, C.D., Anastasaki, E., Tarantilis, P.A., Polissiou, M.G., Alonso, G.L.et al
saffron stigmas	extraction of crocin gentiobiose imprinted polymer (Gent-MIP	crocin, safranal and picrocrocin	Extraction of crocin from saffron (Crocus sativus) using molecularly imprinted polymer solid-phase extraction	Mohajeri, S.A., Hosseinzadeh, H., Keyhanfar, F., Aghamohammadian, J.et al
saffron	Ultrasound-	diethyl	Rapid	Maggi, L.,

spice	assisted extraction of safranal	ether, hexane and chloroform	determination of safranal in the quality control of saffron spice (Crocus sativus L.)	Sánchez, A.M., Carmona, M., Kanakis, C.D., Anastasaki, E., Tarantilis, P.A., Polissiou, M.G., Alonso, G.L.et al
ingredients of saffron	liquid chromatography-UV (HPLC-UV)	Methanol and 1% acetic acid	Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography	Li, N., Lin, G., Kwan, Y.-W., Min, Z.-D.et al
safranal content of saffron	carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography	safranal and HTCC	A non-destructive method to determine the safranal content of saffron (Crocus sativus L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography	Lozano, P., Delgado, D., Gómez, D., Rubio, M., Iborra, J.L.et al
Saffron	saffron extract itself	carotenoids,	Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.)	Abdullaev, F.I.et al

samples of saffron	SPME-GC-MS analysis	3,5,5-trimethyl-2-cyclohexen-1-one, 3,5,5-trimethyl-2-cyclohexen-1,4-dione, safranal and 2,4,4-trimethyl-6-hydroxy-3-carboxaldehyde-2,5-cyclohexadien-1-one were found. 3,5,5-Trimethyl-1,4-cyclohexandione	Volatile organic compounds from saffron	D'Auria, M., Mauriello, G., Rana, G.L.et al
Saffron suspension-cultured cells	HPLC-DAD analysis	Salycilic acid	Salycilic acid induces exudation of crocin and phenolics in saffron suspension-cultured cells	Moradi, A., Zarinkamar, F., De Domenico, S., Mita, G., Di Sansebastiano, G.P., Caretto, S.et al

Table 3: Genetic diversity of saffron by various marker genes.

Species	Title of the paper	DNA based Marker	Development methods	Potential applications	References
Crocus sativus	NIR Specactroscopy multivariate analysis for rapid authentication detection and quantification of common plant adulterants in saffron(crocus sativus L) stigmas.		Partial least squares-discriminant analysis(PLS-DA) model .	Provides a useful quality assessment tool for saffron in an attempt to prevent its fraud.	Shawky et al (2020).

Crocus sativus	Advances in bioactive compounds from crocus sativus(saffron):structure,bioactivity and biotechnology		Tissue culture advance method for large scale production of infection free corm biomass of Crocus	Illustrates therapeupic potential of saffron and its active phytoconstituents ,their extraction method.	Panday,D.K et al (2020)
Crocus sativus	A comperative assessment of DNA fingerprinting assays of ISSR and RAPD markers for molecular diversity of saffron and other crocus spp, in Iran	ISSR and RAPD markers	Principal coordinate analysis of the pooled data of both the ,arkers also support their UPGMA dendrogram	Helps in future genetic improvement programme of corcus species	Najafi Zarini et al (2019)
Corcus sativus L	Molecular and chemical characterization of mutant and non mutant genotypes of saffron grown in Saudi Arabia	PCR-RAPD analysis	An unweighted pair group method mean(UPGMA)	T0 of crocus sativus genotype showed divergence	Sharaf-Eldin et al (2019)
Crocus sativus	Greek PDO saffron authentication studies using species specific molecular markers	Bar-HRM	HPLC-fluorescence determination of secondary metabolites	Bar-HRM approach is quite effective in terms of specificity and sensitivity	Bosmali et al (2018)
Corcus sativus	Genetic diversity of c.sativus and its close relative species analysed by iPBS-retrotransposons	iPBS-retrotransposons markers	UPGMA dendrogram and PCoA analysis	Saffron genotype showed very limited genetic variation	Gedik et al (2017)
Corcus sativus	Molecular authentication and quality control of c.sativus and aloe barbadensis in raw material source and ployherbal medicine	RAPD and SCAR markers	c.sativus may not have been used to prepare the polyherbal	Used for large scale screening of medicinal plants at raw material source as well	Gul et al (2016)

	employing scar markers		medicine	as in finished polyherbal medicine	
Corcus sativus	Analysis of genetic diversity among saffron (c.sativus) accessions from different regions of Iran as revealed by SRAP markers	SRAP markers	PCA showed that cluster analysis is more appropriate for revealing genetic relationship of saffron accession	SRAP markers are powerful tools and effective marker system for evaluation of genetic diversity amoung saffron accession	Babaei et al (2014)
Corcus sativus	Saffron is a monomorphic species as revealed by RAPD, ISSR and microsatellite analyses	RAPD, ISSR and microsatellite analyses	all accessions appear identical clones, not only because morphological characters but also at a molecular level.	genome sequencing is needed to find molecular markers for saffron.	Rubio-Moraga et al
Corcus sativus	Genome-based approaches to the authentication of medicinal plants	polymerase chain reaction	Genomic fingerprinting can differentiate between individuals, species and populations and is useful for the detection of the homogeneity of the samples and presence of	The generation of molecular "barcodes" of medicinal plants will be worth the concerted effort of the medicinal plant	Sucher et al 2008

			adulterants		
Corcus sativus	Analysis of genetic diversity and phylogenetic relationships in crocus genus of iran using inter-retrotransposon amplified polymorphism	(LTR)-retrotransposon primers	The analysis revealed a close relationship between saffron and three wild species	C. almehensis, C. michelosnii as the closest relatives of saffron and probably the possible wild ancestors of this cultivated species	Alavi-Kia et al
Corcus sativus	RAPD analysis in Crocus sativus L. accessions and related Crocus species	Random Amplified Polymorphic DNA (RAPD)	DNAs of saffron corms from different accessions present the same amplification pattern, in accordance with the similar DNA content and base composition pointed out in previous studies. c. Results indicated that C. sativus is very closely related to C. cartwrighianus	concurring with part of the previous evidence, would rule out the hypothesis of close relationships between C. sativus and C. pallasii.	Caiola et al

			and also similar to C. thomasii.		

Conclusion

Earlier molecular studies of saffron evolution failed to reach clear results relating to the parental species of C. sativus or the world of origin of saffron. the most reason looks that they failed to take under consideration the high intra-specific genetic diversity gift in C. cartwrightianus. reckoning on the individual(s) studied and also the marker region used, theresulting organic process trees would possibly mirror nearly arbitrary relationships). In distinction, our GBS knowledge were supported AN thoroughgoing assortment of C. cartwrightianus populations and clearly place the C. sativus people as sister of Attic C. cartwrightianus. potential reasons for the sister cluster position rather than grouping inside the Attic population is maybe the polyploid and organism nature of C. sativus that, as a group, has thus a novel character combination that's during this manner not gift in any person of C. cartwrightianus. Still, overall frequencies of GBS alleles ANd additionally plastid knowledge support an origin of saffront the northwestern distribution limit of C. cartwrightianus with nearest similarity of the crop to the wild plants occurring in dominion.We cannot however confirm what quantity triploidy influences the event of the everyday traits of saffron or if the proper gene combination during a diploid would possibly offer similar characteristics. Still, the clarification of the mode mode of evolution of C. sativus currently parades a route for overcoming the low genetic diversity gift within the saffron, because it can foster new saffron genotypes to be created from totally different C. cartwrightianus people.

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