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VI International Symposium on Saffron Biology and Technology

ORAL PRESENTATIONS

OP 1:

SAFFRON PRODUCTION IN TURKEY

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Turkey located at the crossroads of Balkans, Caucasus, Middle east and eastern Mediterranean (38°57'49.5" N 35°14'36" E). *Crocus* genus is an important crop for Turkey which is represented by nearly 80 species. *Crocus sativus* is one of the most significant taxa in this genus. Stylus of the *C. sativus* known as saffron and this plant cultivated for its spice. It is the most expensive spice in the world. Saffron are used in varied products as Turkish delight and some local food such as zerde dessert, medicines and cosmetics. Saffron is cultured commonly in Safranbolu province in Turkey and annually 30 kg saffron spices produced in Turkey. Besides, at the recent times saffron production are enlarged to different location especially at eastern Taurus Mountains and Southeastern part of Turkey. In this review saffron production in Turkey was investigated and reported.

Keywords: *Crocus sativus*, spice, products, Safranbolu, Turkey

OP 2:

SAFFRON (*Crocus sativus* L.) FROM THE SPANISH REGION OF ARAGON: VALORISATION OF THE QUALITY

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Saffron spice, made up of the dried stigmas of *Crocus sativus* L., is chiefly used in food due to its colouring, flavouring and aromatic properties. Throughout history, the outstanding Spanish saffron quality has been highly valued all over the world and Spain has played a key role in international saffron markets. Aragon in the northeast of Spain was a traditional and important saffron production region with more than 20% of the Spanish output. Within Aragon, the main output was obtained in the valley of the river Jiloca, in the province of Teruel. In the recent years, after a long-term decline of cultivation in Aragon, there has been a renewed interest in saffron as a sustainable high value agricultural product and, therefore in the valorisation of its quality. The objective of this work was to valorise the quality of saffron spice from Aragon by its characterization through ISO 3632 parameters, the study of phytosanitary problems of the crop influencing quality figures, and the analysis of consumer knowledge, purchasing habits and preferences regarding saffron quality. Main characteristics of more than 70 saffron samples from Aragon were determined by the ISO 3632 ultraviolet-visible spectrometric method. Virus and fungi infections were studied in plant material and cultivation soils by biochemical, molecular, and serological tests. Consumer study was carried out through a survey to 202 consumers in 18 working sessions.



Keywords: *Crocus sativus*, colouring strength, crocetin esters, picrocrocin safranal, potyvirus, Fusarium, purchasing habits

OP 3:

GENETIC DIVERSITY AMONG THE CLOSEST RELATIVES OF SAFFRON CROCUS

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We have studied inter- and intraspecific variation in Series *Crocus*. This group comprises 10 species, including the cultivated saffron crocus, *Crocus sativus*. Members of the group are autumn-flowering (October – December), characterized by a deeply 3-divided red to orange stigma and yellow anthers. The flower of *C. cartwrightianus* resembles the flower of *C. sativus* and the two species are therefore supposed to be closely related. During a number of field trips to the Attica Peninsula and the archipelagos (Greece) we have studied the habitats and morphological variation of *C. cartwrightianus*. We also included *C. oreoreticus* from the central and western part of Crete, *C. hadriaticus* from Peloponnese and the mainland of Greece and *C. pallasii* from Southern Greece and Turkey in our studies. The genetic diversity was analysed among nine species of Series *Crocus* using SSR-markers and AFLP. We found a large diversity between and within the studied species and lack of population structure. There was no genetic differentiation between different accessions of *C. cartwrightianus*, *C. oreoreticus* and *C. hadriaticus*. The findings suggest that a huge diversity is kept in wild populations and that considerable gene-flow exist between populations. Further exploration of the closest relatives of *C. sativus* using genome-wide markers should provide new insight in the genetic diversity and provide markers that can facilitate selection of superior genotypes that may bring new variation into cultivated saffron crocus.

Keywords: *Crocus cartwrightianus*, *Crocus oreoreticus*, wild relatives of *Crocus sativus*, habitats, genetic variation, breeding of saffron crocus

OP 4:

THE EXPLANT STORAGE CONDITIONS EFFECT AND THE OPTIMIZATION OF THE MICRO-CORMS PRODUCTION PROTOCOL OF THE SAFFRON (*Crocus sativus*) IN VITRO CULTIVATION

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Saffron is a sterile geophyte that was growing in earlier times in the south-west of the Moroccan High Atlas. Its vegetative propagation by corms constitutes a constraint to the extension of its culture because of its low rate of multiplication, the diseases problems, and the heterogeneity of the seed produced. *In vitro* micropropagation of saffron is an appropriate alternative for mass multiplication of selected seeds. The different protocols documented so far depend on the season and a specific phenological stage for the corms. In order to upgrading to commercial scale propagation, the *in vitro* propagation of saffron needs to be studied closely. A first attempt was to study the effect of different storage times of dormant corms at 35°C on the length of the initiation phase. This first stage of the *in vitro* cultivation was reduced to 3 weeks compared to the 8 weeks reported previously by other authors and obtained with the no stored control. On the other hand, the effect of the storage of corms at



35°C was investigated and was found to maintain the dormancy and the viability of the explants during all the 14 months of experimentation and ensured the initiation of new corms *in vitro* during the four seasons of year regardless of their growth cycle. The optimization of the micropropagation protocol focused on the four micropropagation phases: initiation, budding, multiplication and establishment and magnification of micro-corms. All phases were undertaken on the Murashige and Skoog medium and differed in their hormonal composition and content, sugar and a plant growth retardant (CCC) concentrations and the length of the different phases. The produced vitro-corms weighting over 4 g were planted for 4 weeks on a peat substrate for their sprouting before being transplanted successfully to the field.

Keywords: *Crocus sativus* L, micropropagation phases, storage conditions, explants initiation

OP 5:

ENGLISH SAFFRON - THE REVIVAL OF A LOST INDUSTRY

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Saffron was once grown in England. For hundreds of years the trade flourished. In fact the export demand for this British Spice was so large that a whole town, the centre of both the trade and the traditional growing area, was named after the industry. The cultivation died out very quickly, however, over 200 years ago and the reasons for this are discussed. English Saffron have reintroduced this once traditional spice back to its traditional growing area around Saffron Walden in Eastern England. This paper describes the many challenges we have faced over the years from climate and richer soil conditions, to attack by predators and disease due to the unique growing conditions we have in England as compared with more traditional areas with warmer and far drier climates. Various growing methods have been researched and experimented with and this continues to be an on-going area of our research. English Saffron was reputed to have a different flavour as compared with imported Saffrons from other parts of the world. Through various taste tests by renowned chefs this has proven to be the case and the reasons for it are discussed.

Keywords: England, Walden, UK, taste, soils

OP 6:

RECENT TECHNIQUES USED FOR SAFFRON QUALITY ANALYSIS: A REVIEW

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Saffron, the dried stigmas of *Crocus sativus* L. has a long history of being used a spice for flavoring and coloring food preparations, as well in traditional medicine, it has been reputed to be efficacious for the alleviation and treatment of ailments. Saffron quality is determined by its purity and its coloring, aromatic and taste properties. The compounds respectively responsible for these properties are crocins, safranal, and picrocrocin. These compounds were measured by spectrophotometry in the UV-vis range, analytical method imposed by ISO standard. It is quick and easy to implement, but it does not allow actual quantification of safranal and picrocrocin. Most of the studies have been carried out on the development of analytical methods related to this quality. This review article covers current analytical techniques, instruments and methodologies used, comparing between them and concluding the most appropriate from an efficiency and economical point of view.



Keywords: Saffron quality, *Crocus sativus* L. analytical techniques, crocins, safranal, picrocrocin, extractions.

OP 7:

ARBUSCULAR MYCORRHIZAL ASSOCIATION PROMOTES *Crocus sativus* MULTIPLICATION UNDER POT CULTURE CONDITIONS

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Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbiotic associations with the roots of living host plants. AMF enhance plant nutrient and water uptake and can act as biofertilizers. There is limited information on AMF association with saffron roots and their effect on agronomic performance. We analyzed the effect of AMF inoculation on sterile sand pot cultivations of saffron. Two inocula (one with a single fungus *Rhizophagus intraradices* and one with *R. intraradices* and *Funneliformis mosseae*) were applied and compared to a control and a mock inoculation. AMF occurrence was characterized through optical and electron transmission microscopy during the vegetative period, together with mortality rate and corm growth. High AMF colonization levels (>70%) and arbuscule production (>59%) were recorded in inoculated pots while little or no colonization occurred in control and mock pots. AMF symbiosis did not influence corm growth (diameter and weight), but increased the production of daughter corms and reduced the occurrence of fungal diseases (ca -72%), compared to control.

Keywords: AMF, saffron, *Rhizophagus intraradices*, *Funneliformis mosseae*, root colonization

OP 8:

QUALITY OF SAFFRON SPICE: COLORING STRENGTH

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The quality of saffron, the dried stigmas of *Crocus sativus* L. is evaluated through various criteria that depend on its chemical composition and the processes it undergoes which can alter it. Research into the chemical composition of saffron has seen a renaissance in recent years. Saffron color has received much attention from scientists due to its bioactivity as well for its natural dye, which has practically disappeared with the development of synthetic coloring. Consumers are avoiding foods containing synthetic colorants, which lead food industries to replace them by natural pigments. Different analytical methods have been established, enabling rapid authenticity control of the spice. Quality established by chemical composition, physicochemical and microbiological properties, cannot be determined by the consumer. They can only be known through analytical determinations. The consumer perceives is influenced by the aspect, basically color, which is what determines the degree of acceptance or rejection. Then, a second evaluation is made on saffron's organoleptic



properties, its flavor and aroma. These characteristics hold increasing greater specific weight in the global idea of saffron quality, but in spite of it they continue subordinated to color. The objective of this review article is to provide a recent development in the chemistry and analysis of saffron color, results that may help to delimit the research for future studies.

Keywords: *Crocus sativus* L., saffron quality, food colorants kinetics, crocins, crocetin esters, carotenoids

OP 9:

INFLUENCE OF INTEGRATED NUTRIENT MANAGEMENT ON STIGMA AND CORM YIELD OF SAFFRON (*Crocus sativus* L.)

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Using Integrated nutrient management module (INM) concurrently may not only quench the crop nutritive demands but also been proved an eco friendly nutrient source. In order to evaluate the effect of nutrient application from INM sources on saffron, a field experiment was conducted on silt clay soil at Saffron Research Station, Pampore SKUAST-K during khraif season 2014-17 to evaluate the response of integrated nutrient management on Stigma and corm yield of saffron (*Crocus sativus* L.). The experiment was conducted in factorial randomized block design with three replications. The levels of fertilizers were 90, 120, and 150 kg ha⁻¹ of nitrogen; 10, 15, and 20 tons ha⁻¹ of farm yard manure (FYM), and 05 and 10 quintal ha⁻¹ of vermicompost. Evaluation over 4 years confirmed that saffron responded to organic manures when used in integration with inorganic fertilizers. Application of 120 kg N ha⁻¹ in conjunction with 15 t farmyard manure (FYM) ha⁻¹ plus Vermicompost 10 quintal recorded highest stigma yield and exhibited 57% increase over control. The combined application of fertilizer N, FYM and Vermicompost sustained the productivity even at lower rate of fertilizer N application. Moreover, the soil organic carbon, available N, P and K content increased with the application of fertilizer N, P and K alone and in conjunction with organics (FYM, vermicompost) compared with control. Integrated nutrient management module with plant population load of 12 lakh corms/ha fetch returns to the tune of Rs 1,02,05,345 on account yield of 41.32 kg saffron/ha and 242 quintals of corms/ha over a period of 4 years. Average productivity of 10.95 kg/ha was observed when Nitrogen 120 kg, FYM 15 tons and Vermicompost 10 quintal was applied ha⁻¹. FYM and Vermicompost application significantly increased both flower yield and corm production thus it can be effectively used as an alternative for application of FYM which presently is unavailable and if available is very costly. Moreover, given years, the use of integrated nutrient management module earned maximum benefit cost ratio of 4.15:1. Conclusively, integrated nutrient management module might be a better technique over separate application of inorganic source.

Keywords: corm, FYM, nitrogen, vermicompost, yield,

OP 10:

CORM WEIGHT AND PLANTING DENSITY EFFECTS ON STIGMA AND CORM YIELD IN TEMPERATE SAFFRON (*Crocus sativus* L.) PRODUCTION

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Saffron (*Crocus sativus* L.) production is gradually spreading in both traditional and nontraditional areas of Jammu & Kashmir India, where production is limited by erratic, inadequate rainfall and inappropriate corm weight and planting density. To increase saffron yield, production practices should be properly designed to minimize the effects of low precipitation, fluctuation in day/ night temperatures, relative humidity and improper corm weight and planting density observed in the last couple of years in saffron growing districts of J&K India warranted to study effects of corm weight and planting density on saffron under prevailing changing climatic pattern. Therefore a study was initiated in 2016 to develop profitable production system module at Saffron Research Station, Pampore SKUAST-Kashmir to determine a suitable corm weight and planting density for saffron, involving two corm sizes based on the corm weight in two levels ($W_1 = 6-7g$ and $W_2 = >7g$) with 6 densities viz: 18 lakh corm density/ha, 15 lakh corm density/ha, 12 lakh corm density/ha, 10 lakh corm density/ha, 05 lakh corm density/ha and 03 lakh corm density/ha (farmers practice). Except farmers practice, saffron corms were planted in ditches of diverse plant geometry to accommodate 05 unlike densities. Traits including plant emergence percentage, number of plants and flowers per unit area, length of rod, length of stigma, fresh and dry weight of flowers, fresh and dry weight of stigmas, total stigma yield, corm multiplication ratio, the onset of flowering and flowering period were evaluated on the plants. Results showed that all above mentioned traits except length of rod were significantly affected by corm weight and planting density. Flowering of bigger corms initiated earlier and their flowering period was more than others. Evaluation over 4 years confirmed distinct yield superiority by planting corms $>7g$ with plant population of 12 lakh corms/ha on raised bed fetch returns to the tune of Rs 1,05,03,000 on account sale of 47.52 kg saffron/ha and 250 quintals of corms/ha with B:C ratio of 4.28:1 over a period of 4 years.

Keywords: Climatic, Corm, density, flowering, geometry, stigma, weight, yield



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POSTER PRESENTATIONS

PP 1:

IN VITRO REGENERATION OF SAFFRON IN THE WORLD

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Crocus sativus L. known as saffron is one of the important crop due to its medicinal and aromatic properties. This species distributed across the Mediterranean basin. Stigmas of the saffron are used as spice in different industry for instance dye, medicine and cuisine industry. Important producers of saffron are Iran, Spain, India, Greece in the world. Saffron is male sterile plant thus corms are produced by daughter corms and tissue culture techniques. Breeding is nearly impossible for this species because of male sterility. Therefore, tissue culture methods such as micropropagation, somatic embryogenesis, organogenesis are very effective for clonal propagation of saffron. Moreover in vitro mutation breeding, nuclear fusion, gene transformation are alternative method to develop new varieties and breeding for saffron. Most of the studies are focused on organogenesis of saffron to obtain mass production. In this review, all tissue culture researches are investigated in detailed for saffron.

Keywords : *Crocus sativus*, spice, stigma, production, tissue culture

PP 2:

STABILITY OF SAFFRON EXTRACTS AT DIFFERENT PH

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Saffron spice (*Crocus sativus* L.) plays an important role in cookery and food industry due to its coloring properties, pleasant bitter taste and characteristic aroma. This is because of the main compounds of saffron: crocetin esters, picrocrocin and safranal. The relevance of this spice generates that several studies on the stability of saffron have already been performed. Thus, effect of different storage conditions on the stability of the main saffron compounds have corresponded to an overall kinetic of first order for crocin and second order for picrocrocin. The results obtained so far are interesting for the food industry, but to our knowledge, there are not assays regarding the effect of pH on the main saffron compounds. The aim of this work was to study the stability of the main compounds of saffron extracts during storage at 35°C ± 2°C, different pHs and lightness and darkness conditions. Saffron extracts at pH: 3, 4, 5, 6, 7 and 8, plus a control extract (pH between 5,50 and 6,00) were kept at a constant temperature (35°C ± 2°C), being subjected to lightness and darkness conditions. Daily, commercial quality parameters were analyzed by ultraviolet-visible spectroscopy and the concentration of each of the main compounds was obtained by HPLC-DAD. Data corresponding to the crocetin esters showed better fits in a first-order kinetic model than in a zero-order or second-order and the results of picrocrocin corresponded to an overall kinetic of second-order. Kinetic parameters varied with the pH, being pH 6 and 7 the most stable. No significant differences were found between the extracts exposed to light and darkness.

Keywords: Saffron, *Crocus sativus* L., kinetics, crocetin esters, picrocrocin



PP 3:

HOW WE COULD HELP IRANIAN SAFFRON PRODUCERS TO INCREASE THEIR YIELD PER HECTARE

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Saffron is one of the most expensive spices and Iran is the main saffron producer in the world. Farmers must pay attention to several options for having good yield, one of them is using fertilizers on time and in specified amount. In order to this fact, we performed a fertilizing Project collaboratively with Green Has. Italy Company in Mashhad, Iran. This pilot performed as completely accidental block with two variable factor: 1) 14 nutritional programs content 4 stages accompany with irrigation and 3 stages foliar application 2) 4 group of different weight corms (1-4gr, 4-8gr, 8-12gr, and 12-16gr). We investigate 9 object for two years: leaves length, flowers number, dry stigma's weight, and wet stigma's weight, stigma's length, and daughter corm's weight, number of daughter corms, daughter corm's diameter, flower's weight and analyzed data with Minitab software. All qualities are shown meaningful expressive with control. Two qualities are more important between them. First one is dry stigma's performance the index of economic yield which 3 and 6 programs are shown more differences according to control. Second one is daughter corm's weight the index of next year yield which 10 and 3 programs are shown more differences with control.

Key word: saffron, Iranian producers, increase, yield, fertilizers.

