Room Table: Agronomical and Biotechnological Approaches for Saffron Improvement

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On behalf of panel members, it is a pleasure and an honor for me to speak to you today, as chairman of the Room Table "Agronomical and Biotechnological Approaches for Saffron Improvement", last element of the second session (Saffron Crop Techniques, Breeding and Tissue Culture) of the 1st International Symposium on Saffron Biology and Biotechnology held in Albacete, Spain.

First of all, I would like to congratulate Prof. José-Antonio Fernández for the organization of such a well-organized symposium and to introduce you the speakers of the room table (Table1).

Dear participants,

In this symposium many of the papers presented up to now, and more papers will be present afterwards, mainly deal with reproductive biology, growth regulation, biosynthesis pathways, cultivation, mutagenesis, commercial and medicinal uses, chemical composition, anticancer effects, etc., of saffron plant. At this point I would like to make brief review about saffron as opening remarks of this room table.

OPENING REMARKS

Crocus sativus L., the best-known species of genus *Crocus,* is cultivated for its spice for at least 3,500 years (Plessner et al., 1989). Odor (safranal), taste (picrocrocin) and pigment (crocin) components, constituting the spice "saffron", are localized in the red stigmatic lobes of the *C. sativus* flower (Himeno and Sano, 1987; Negbi et al., 1989; Plessner et al., 1989). It has a sweetish aromatic odor and a bitter taste (Trease and Evans, 1983). Saffron was also used for treating many diseases (Basker and Negbi, 1983; Giaccio, 1990). It may stimulate appetite and prevent gastro-intestinal atony. The principal active substances present in saffron spice are crocin, picrocrocin and safranal (Escribano et al., 1996). Crocin affects on uterine muscle contractility. It has been pointed out that increases blood oxygenation by accelerating its transport speed. Picrocrocin has a sedative effect on spasms (Giaccio, 1990). Today saffron is noted as a spice, adding its faint, delicate aroma, pleasing flavor and magnificent yellow color to enhance palatability. The range of foods that have been spiced with saffron is wide, including cream or cottage cheese, chicken and meat, rice, cakes, mayonnaise, liqueurs, etc. (Basker and Negbi, 1983). Some researchers have also studied the pharmacological properties of saffron. Basker and Negbi (1983) stated that saffron is the richest known source of riboflavin. The antitumor activity of saffron extract against the growth of tumor cells has been investigated (Nair et al., 1991; Escribano et al., 1996; García-Olmo et al., 1999; Premkumar et al., 2001; Abdullaev, 2002). They indicated that saffron might be a potential anticancer agent. Liakopoulou-Kyriakides and Skubas (1985,1990) found that bulbs of *C. sativus* contain two protein factors with aggregating and inhibitory properties on human platelets. Researches on the antitumoral properties of corm components of saffron plant have also been carried out (Escribano et al., 1999a,b).

Reproduction

C. sativus can be propagated only by its corms because its sterility. *C. sativus* is an autotriploid plant showing eight trivalents in its meiosis (Chichiricco, 1984). Its sterility is due to its autotriploidy (Ghaffari, 1986) that causes meiotic irregularities in both sporogenesis and gametogenesis. As a consequence, most of the pollen grains show abnormalities and sterility (Chichiricco and Grilli Caiola, 1982; Chichiricco, 1989) and the ovules show an unfunctional embryo sac (Chichiricco, 1987). Nevertheless, after stigmatic pollination, a number of pollen grains develop tubes that extend through the

style to the ovary but fail to penetrate the ovules (Chichiricco, 1990).

In addition, pollen dehydrates after flowers opening and loses their germinating ability within three weeks from shedding (Chichiricco, 2000). Pollen has cytological abnormalities and germinates in low percentage in all stigmas and some pollen tubes grow down to the ovules (Chichiricco and Grilli Caiola 1984) [see Grilli Caiola in chapter 1 of these Acta for more detailed information].

C. sativus Propagation and Cultural Techniques

Most of the *Crocus* species grow naturally. Only a few *Crocus* species are being cultivated and produced on a commercial scale. Among these, *C. sativus* has a considerable place as mentioned. Traditionally, its corms are planted in soil August or September. The flowering takes place in October or November. Each corm replaces itself by one or more daughter corms. After harvesting of flowers, corms, which have at least doubled in number, are dug up in May or June (Vurdu et al., 2002a).

C. sativus corms grow best in a calcareous and well-drained soil that has fairly loose texture and permits easy root penetration. Acid and high pH soils are unsuitable. Although temperature and humidity requirements are not rigid, saffron crocus grows better in warm or subtropical climates. Corm diameter was found to be the most influential factor for the number of daughter corms and flower yield (Vurdu et al. 2002b). Mother corms die after flowering period and two or more corms develop to replace the older ones. This process continues for several years and every year the corms rise about two cm higher in the soil from those of the previous year and finally they reach soil surface.

Saffron is a slow growth geophyte and depends on vegetative propagation. This opens the way to fungi and insect infestation (Negbi et al., 1989; Vurdu et al., 2002a). A fungus *Fusarium* sp., pathogen of saffron plant, has been observed (Vurdu et al., 2002a). Coleopteran larva that is giving harmful damage to the saffron corm was also detected (Şaltu, 2002). For the new plantation, well-developed and healthy new corms should be used. Negbi et al. (1989) stored saffron corms successfully under uncontrolled ambient conditions after collecting in the field and spread them over nets, and latterly keeping them in brown paper bags. Before planting, *C. sativus* corms must be disinfected with penta chloro-nitro benzene (PCNB) or brassicol or copper sulfate ($CuSO_4$) (Skrubis, 1990). To go deepen in the propagation of *Crocus*, Negbi et al. (1989) suggest the immersion of corms in an aqueous mixture containing 13 g/l Benlete (against *Rhizoctinia violacea, R. crocorum,* and other pathogenic fungi) and 0.6 g/l Dexon (against mealybugs, for 30 minutes. Likewise, the effects of growth hormones on saffron growth characteristics have been studied (Vurdu et al., 1997). Corms treated with a concentration 50 mg/l of synthetic hormones auxin (PS-Ab) and cytokinin (PS-K), for a period of three hours, germinated earlier and produced more sprouts and daughter corms (Vurdu and Allahverdiev, 1996; Vurdu et al., 1997).

In conventional saffron cultural techniques, soil is cleared of weeds and other undesirable materials for corm planting. The land is ploughed three times before planting. The first ploughing is done to a depth of 30 to 35 cm in March. The second is made about three weeks later at a depth of 20 to 25 cm. At this time, some farmers use farmyard manure (20 tons/ha) and mix it thoroughly with the soil. The third ploughing is done a few days before corm planting (Negbi et al., 1989). At this stage, adding the cattle manure to the soil by ploughing is recommended (Şaltu, 2002). Negbi et al. (1989) recommended that corms should be planted at a depth of 10 to 15 cm, in rows 20 cm apart, and with a distance of 10 cm within rows.

Saffron production requires abundant labor. Each worker can pick up about 15,000 flowers per day that corresponds to 100 g of dried stigma (Skrubis, 1990). The collection of flowers must be done carefully to facilitate separation of petals from the stigmas and stamens. Therefore, saffron production is carried out manually (Trease and Evans, 1983; Negbi et al., 1989; Plessner et al., 1989) and is laborious. The increasing of labor costs since the 1960s led to a reduction of saffron acreage in Turkey and Spain, and to the

almost complete disappearance of this crop in Italy. During these decades, there was, however, an appreciable increase in the northern Greece, where saffron is cultivated co-operatively in and around the village Krokos (Negbi et al, 1989; Plessner et al., 1989). A reduction in the production costs would be achieved by mechanical harvesting of *C. sativus* flowers. Mechanical harvesting, however, is hindered by two factors (Plessner et al., 1989):

i) Flowers grow a few centimeters above the soil surface,
ii) Flowering usually takes place with/or after leaf appearance.

Hence, mechanical harvesting of the flowers would damage the foliage and then drastically reduce the production of replacement corms, which are indispensable for the propagation of this sterile taxon. Plessner et al. (1989) studying the improvement of the cultivation methods, demonstrated that controlled environmental conditions during corm storage and planting, affect flowering and promote appearance of flowers earlier than leaves. If early flowering is achieved on a commercial agricultural scale, this would increase the efficiency and competitiveness of saffron production [see Molina et al., in chapter 25 of these Acta for additional information].

Turkey was used to be a saffron producer country in the past. However, the cultivation of saffron is almost coming to end in Turkey. Nowadays, there is only a couple of farmers who have been cultivating saffron in a very restricted area of Safranbolu (Vurdu, 1993). The major reasons are;

i) difficulty at the saffron marketing for domestic and international markets causing lower income to the producers and,
ii) fast migration from rural areas to cities for better standards of life and benefit, resulting that almost all of the young people left the villages.

Because of the high price of saffron spice, it has been the object of multiple forms of adulteration. Even thought the International Standard Organization (ISO) established standards, specifications and test methods for saffron spice (ISO 3632-1 and ISO 3632-2) adulteration does not seem to be have abated so far. Thus, traditional propagation and harvesting process, as well as the related international standards for quality, need further developments to be carried out.

In Vitro Propagation

Production in vitro of corms of *C. sativus* is aimed to the improvement of saffron spice yield. The use of tissue culture techniques has been assayed in *C. sativus* with limited success. Ilahi et al. (1987) reported plantlets developed on calli obtained from corms, but no rooting occurred at the experimental temperature of 25^{o} C. They detected bud development from the cut surfaces of corms. 2,4-D and zeatin were found to be essential for regular development of *C. sativus* explants and enhanced bud development on intact corms in vitro. Ethylene pretreatment also induced corm production (Plessner et al., 1990). Callus induced by 2,4-D and zeatin developed small spherical organ-like nodules, which generated into shoots after 12 weeks (Isa and Osagawa, 1988). Plant tissue culture is also promising as an alternative to the whole plant for the production of the spice saffron (Sarma et al., 1990). Stigma-like structures were produced in tissue culture from stigma explants of *C. sativus*. Crocin and picrocrocin pigments responsible for color and bitter taste, respectively, were extracted identified and quantified from the TC stigmas. Safranal was not detected in fresh sample.

Fakhrai and Evans (1990) reported that all the floral parts of the *C. sativus* have the potential for the production of stigma-like structures. The ovaries of *C. sativus* are induced to fructify if growth-regulators (2,4-D, GA3 and BAP) are added singly or in combination to the medium. However, all the fruits are parthenocarpic and they are turgid and oblong-shaped (Chichiricco and Caiola, 1987).

The continuous tissue culture techniques shall be well developed in order to improve the production of the spice by large-scale propagation of selected pathogen-free ecotypes and the potential application of gene transfer to saffron plant (Piqueras et al., 1999). Corm is known to be infested by pathogenic fungi and viruses, which are

transferred by the corms to the next generation (Plessner et al., 1990). In conclusion, in vitro propagation techniques of pathogen-free corms need to be developed using tissue culture techniques (see references in Table 2).

I guess that I did not take your time too much. I thank you very much for listening my opening remarks with a kind patience. Now the room table speakers will discuss, giving their points of view with regard to the agronomical and biotechnological approaches for saffron improvement. Then, I am expecting the participation of the audience in the discussion.

DISCUSSION

After the opening remarks have been given, the main points of the discussion of the speakers and the participants are summarized.

Dr. Abel Piqueras pointed out that "Corm quality needs to be improved. For this, there is a complement or a choice between conventional and tissue culture techniques. For the time being, the application of tissue culture has several problems including growth inhibitor, availability of stem meristem in a very limited time, etc. And thus, it needs further development".

Contrary, Prof. Alireza Koocheki stated that "enough saffron has been produced; 150 tons in Iran and thus expanding saffron production and improving tissue culture techniques cannot be acceptable for Iranian because of their intellectual property rights". His view can be considered as a monopolist approach. In addition, the mentioning "rights", if there are any, cannot be protected by ignoring the development of in vitro propagation.

Prof. José L. Guardiola stated that "there are some difficulties getting local literature related to saffron from the undeveloped countries". He also emphasized the need of mechanization in saffron production.

Mr. Hossein Fekrat (Tarvand Saffron Co., Iran) and Dr. Jürgen Rohmeder (Saffranerie Gmbh, Switzerland), from the audience, discussed about the quality of saffron and the problems of quality control methods. "The current international standards (ISO) related to saffron quality do not work satisfactory and need to be improved".

Room Table Speakers and participants from the audience discussed about different aspects of saffron productions. These perspectives include many different areas e.g. traditional harvesting and mechanization, corm quality and diseases, genetic variability and the application of gene transfer techniques, in vitro propagation and the problems of tissue culture, information about the socio-economic value of saffron, adulteration of spice and the current international regulation system, ISO standards and marketing, consumer protection and standard quality testing methods, traditional use of saffron and the development of medicinal use alternatives, amongst others. They all coincide in the need to intensify research on these matters.

As a conclusion, the following three major scientific and technical goals for the coming years were agreed:

i) Corm planting, flower harvesting, stigma separation, drying, packaging and corm lifting need to be mechanized,
ii) The existing ISO standards shall be simplified for the consumer in order to control quality and avoid adulteration in the spice, and
iii) In vitro propagation of saffron plant, including corm tissue culture techniques, need to be well developed.

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Participants

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Tables

Variety/ mutants	days to sprouting	Days to flowering	Plant height (cm)	Flowering plants (%)	Flower diameter (cm)	Stigma length(cm)	100 stigma weight (g)	Corm yield (per plant)
Parental variety (Kashmira)	47.00	65.00	13.50	16.00	4.30	2.80	0.675	5.20
3-petal mutant	48.00	66.00	13.60	4.00	4.28	2.70	0.670	5.25
Serrated petal mutant	50.00	68.00	14.25	41.00	4.87	2.91	0.688	5.30