Introduction

*Crocus sativus* L. has been cultivated since ancient times as the source of saffron. This is possibly the most expensive spice on earth, which consists of the dried stigmas of *C. sativus*, and is well-known for medicinal and flavoring properties. *Crocus sativus* is a triploid species (Mathew, 1977; Ghaffari, 1986), which is self- and out-sterile (Grilli Caiola, 2005). The plant is therefore unable to produce seeds and must necessarily be propagated via corms. Sterility depends on an irregular triploid meiosis, resulting in many anomalies in sporogenesis and gametophyte development (Chichiriccò, 1999; Grilli Caiola, 2004) which lead to the production of abnormal pollen and self-sterile pollination. Though the occurrence of seeds in the field has been reported only once (Piccoli, 1932; Grilli Caiola, 2004), the in vitro cross-pollination (fertilisation) of the ovaries of *C. sativus* with pollen of *C. cartwrightianus* (Grilli Caiola, 2005; Grilli Caiola et al., 2010) and *C. thomasii* Ten. (a self-incompatible, but cross-fertile species) (Chichiriccò, 1999), has resulted in the production of capsules and viable seeds. *Crocus hadriaticus* is also able to fertilise *C. sativus* (Chichiriccò, 1996), while the pollination of other *Crocus* species with pollen of *C. sativus* did not result in the production of any seeds (Grilli Caiola, 2005).

The genetic origin of *C. sativus* is yet to be ascertained: it may have occurred by autotriploidy from a wild *Crocus*, probably by fertilisation of diploid unreduced egg cells by haploid sperm cells or from haploid egg cells, each fertilised by two haploid...
sperms (Grilli Caiola, 2004, 2005), or by allopolyploidy through the hybridisation of *C. cartwrightianus* and *C. hadriaticus* (Castillo et al., 2005; Grilli Caiola and Canini, 2010). Regarding saffron ancestors, Brighton (1977) in a karyological study suggested *C. cartwrightianus* as the most likely ancestor (Brandizzi and Grilli Caiola, 1998; Grilli Caiola et al., 2004, Grilli Caiola and Canini, 2010). Amplified Fragment Length Polymorphism analysis confirmed that the quantitative and qualitative traits of DNA from these species are compatible with *C. sativus* (Zubor et al., 2004). Furthermore, flowering in *C. cartwrightianus* very likely resembles that observed in *C. sativus*. Brighton (1977) asserted that saffron shows homogenous and stable biological characters all over the world, slightly diverging only in some morphological and biochemical features (Tammaro, 1990). Indeed, Random Amplified Polymorphic DNA investigations on *C. sativus* DNA from five diverse locations (in Europe and Israel) did not identify any genomic differences, though the respective plants showed distinct morphological properties (Grilli Caiola et al., 2004).

**Evidence of latent potyvirus infections in *Crocus spp.***

In the last decades we have ultrastructurally examined stigmas, styles and leaves of more than fifty plants of *C. sativus*, *C. cartwrightianus* and *C. thomasii* from different provenance. Though all the collected plants were apparently healthy, in about 60% of them we found cytoplasmic inclusions typical of potyvirus infection in both *C. sativus* and *C. cartwrightianus*, but never in the wild species *C. thomasii* (Grilli Caiola 1982; Grilli Caiola, unpublished) (Table 1, Figure 1). In particular, the most frequent inclusions observed by transmission electron microscopy, after conventional fixation in 3% glutaraldehyde followed by 1% osmium tetroxide and embedding in Spurr or Araldite (Grilli Caiola, 1982), were pinwheel bundles associated with laminated aggregates and tubular structures. These are the typical features of type-2 potyviruses, according to Edwardson and Christie’s (1978) classification. Considering that a representative member of this potyvirus group is Bean yellow mosaic virus (BYMV) (Hull, 2002), and that this virus has been frequently found in *C. sativus* by different authors (Table 2), it is very likely that BYMV was one of the main viruses responsible of the observed latent virus infections also in our samples. It is noteworthy that inclusions typical of BYMV in symptomless *C. sativus* had already been reported by Russo et al. (1979), confirming that this, and possibly other potyviruses, may cause latent infections in this species. Nevertheless, the presence of other potyviruses, at least in our samples of *C. cartwrightianus*, is suggested by the presence of scrolls, which are typical inclusions induced by type I, III and IV potyviruses according to Edwardson and Christie’s (1978) classification.

A similar multiple potyvirus infection in *C. sativus* was reported by Pisi and Bellardi (1990), who found, besides BYMV inclusions, also short and

<table>
<thead>
<tr>
<th><em>Crocus</em> species</th>
<th>Provenance</th>
<th>Tissue examined</th>
<th>Potyvirus inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. sativus</em></td>
<td>L’Aquila province, Italy</td>
<td>Stigmas and leaves</td>
<td>Pinwheels, laminated aggregates, crystalline bodies</td>
</tr>
<tr>
<td><em>C. cartwrightianus</em></td>
<td>Botanical Garden, Amsterdam, Netherlands</td>
<td>Stigmas</td>
<td>Pinwheels, laminated aggregates, crystalline bodies, scrolls</td>
</tr>
<tr>
<td>(white flowers)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. cartwrightianus</em></td>
<td>England, UK</td>
<td>Stigmas</td>
<td>Pinwheels, laminated aggregates</td>
</tr>
<tr>
<td>(violet flowers)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. thomasii</em></td>
<td>Castel del Monte (Bari), Italy</td>
<td>Gynoecia and leaves</td>
<td>No inclusions</td>
</tr>
</tbody>
</table>
Figure 1. Potyvirus cytoplasmic inclusions in stigmas of *Crocus sativus* (a-c) and *C. cartwrightianus* (d-f) from symptomless plants. L, laminated structures; P, pinwheels; S, scrolls; C, crystalline bodies; T, tubular structures; V, virus-like particles.

<table>
<thead>
<tr>
<th><em>Crocus</em> species</th>
<th>Origin</th>
<th>Virus</th>
<th>Symptom</th>
<th>Examined organs/tissues</th>
<th>Cytopathology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not specified</td>
<td>Denmark</td>
<td>TRV</td>
<td>Yellow/brown oval necrotic spots on leaves, small flowers with color breaking</td>
<td>Not specified</td>
<td>Not examined</td>
<td>Van Slogtern, 1958</td>
</tr>
<tr>
<td><em>C. sativus</em></td>
<td>Siena province, Italy</td>
<td>BYMV</td>
<td>Symptomless</td>
<td>Leaves, roots</td>
<td>Pinwheels, laminated structures, crystalline bodies</td>
<td>Russo <em>et al.</em>, 1979</td>
</tr>
<tr>
<td><em>C. tomasianus</em></td>
<td>Netherlands</td>
<td>ISMV</td>
<td>Stunting, leaf chlorosis, petal color breaking</td>
<td>Flowers, leaves</td>
<td>Not examined</td>
<td>Brunt and Phillips, 1979</td>
</tr>
<tr>
<td><em>C. sativus</em></td>
<td>L’Aquila province, Italy</td>
<td>Potyvirus (BYMV?)</td>
<td>Symptomless</td>
<td>Stigmas</td>
<td>Pinwheels, laminated aggregates, crystalline bodies</td>
<td>Grilli Caiola, 1982</td>
</tr>
<tr>
<td>Not specified</td>
<td>North Europe</td>
<td>CMV, Y-C</td>
<td>Mosaic, retarded growth</td>
<td>Leaves, flowers</td>
<td>Not examined</td>
<td>Bellardi and Pisi, 1987</td>
</tr>
<tr>
<td>Not specified</td>
<td>North Europe</td>
<td>BYMV</td>
<td>Not specified</td>
<td>Leaves</td>
<td>Pinwheels, laminated aggregates, crystalline bodies</td>
<td>Pisi and Bellardi, 1990</td>
</tr>
<tr>
<td><em>C. sativus</em></td>
<td>East China</td>
<td>TuMV</td>
<td>Mosaic</td>
<td>Not specified</td>
<td>Laminated aggregates, scrolls</td>
<td>Chen and Chen, 2000; Jishuang, 2000; Chen <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>Not specified</td>
<td>Nagoya, Japan</td>
<td>BYMV</td>
<td>Not specified</td>
<td>Leaves</td>
<td>Pinwheels, laminated aggregates, crystalline bodies</td>
<td>Kaneshige <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>Not specified</td>
<td>Lithuania</td>
<td>TRV</td>
<td>Yellow/brown oval necrotic spots on leaves, small flowers with color breaking</td>
<td>Leaves and flowers</td>
<td>Not examined</td>
<td>Navalinskiene and Samuitiene, 2001</td>
</tr>
<tr>
<td>Not specified</td>
<td>Lithuania</td>
<td>IMMV</td>
<td>Narrowed, twisted with greenish/necrotic spots and streaks; petals color breaking and crinkled</td>
<td>Leaves and flowers</td>
<td>Not examined</td>
<td>Navalinskiene and Samuitiene, 2001</td>
</tr>
<tr>
<td><em>C. flavus</em></td>
<td>Breezeland and Lisse districts, Netherlands</td>
<td>ISMV, BYMV, TNV, TRV, NMV</td>
<td>Color breaking, stunting, yellowing, necrosis</td>
<td>Flowers, leaves</td>
<td>Not examined</td>
<td>Miglino <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><em>C. sativus</em></td>
<td>New Zealand</td>
<td>TuMV</td>
<td>Chlorosis, mild to severe mosaic, necrosis</td>
<td>Leaves</td>
<td>Potyvirus-like particles</td>
<td>Ochoa Corona <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>C. sativus</em></td>
<td>Porchères, France</td>
<td>Potyviruses</td>
<td>Symptomless</td>
<td>Stigmas</td>
<td>Not examined</td>
<td>D’Agostino <em>et al.</em>, 2007 (Bouvier <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td>Not specified</td>
<td>Lithuania</td>
<td>CMV</td>
<td>Not specified</td>
<td>Flowers</td>
<td>Not examined</td>
<td>Samuitiene and Navalinskiene, 2008</td>
</tr>
<tr>
<td><em>C. chrisanthus</em></td>
<td>Lithuania</td>
<td>ArMV + TRV, + TSWV + TNV (mixed)</td>
<td>Dwarfing, chlorotic streaks, necrotic spots</td>
<td>Leaves, flowers</td>
<td>Not examined</td>
<td>Samuitiene <em>et al.</em>, 2008</td>
</tr>
</tbody>
</table>
Virus infections in Crocus species

curved laminated aggregates characteristic of potyviruses belonging to subdivision IV of Edwardson and Christie (1983, 1984). Unfortunately, the authors did not specify if the plants were symptomless or not, though in a previous paper (Bellardi and Pisi, 1987), possibly referring to the same plants, they reported that the four plants containing potyviruses, out of the 50 examined from an imported stock of corms, were symptomless.

D’Agostino et al. (2007), in an Expressed Sequence Tags database from a saffron stigma cDNA library coming from a symptomless plant collected in France (Bouvier et al., 2003) identified four tentative consensus sequences, Cl000209:1 (61 ESTs), Cl000582:1 (18 ESTs), Cl001827:1 (5 ESTs) and Cl000731 (2 ESTs), showing similarity to potyviral sequences. This finding, besides indicating that the sequenced library likely derived from a plant with latent potyvirus infection, confirms the widespread occurrence of such viruses in cultivated saffron.

The presence of potyvirus latent infections in C. cartwrightianus, a species probably progenitor of saffron (Grilli Caiola and Canini, 2010), deserves particular attention. This could mean that these viruses have been infecting Crocus spp. since ancient times, and that mild virus strains have been selected during host-pathogen co-evolution. Furthermore, the transmission through reproductive organs from C. cartwrightianus to C. sativus cannot be excluded, as in the Potyviridae family virus transmission by pollen to pollinated plants can occur (Mink, 1993). On the other hand, potyviruses such as BYMV are widely diffused in plants in the Iridaceae (Tsuji et al., 1996) and they are aphid transmitted (Hull, 2002). Infections could, therefore, also occur through aphid vectors. However, the fact that we have never found signs of potyvirus infection in the wild species C. thomasii weakens this hypothesis.

The exclusive vegetative propagation of C sativus through corms, besides being the main likely way of spreading viruses, has also possibly determined the selection of potyvirus strains with low aggressiveness, responsible for mild or latent infections. Moreover, the morphological traits of Crocus flowers make it difficult to visually ascertain mild symptoms of stunting or petal colour breaking, often associated to potyvirus infection, and this may also favour virus spread in C. sativus crops.

Other plant viruses infecting Crocus spp.

Besides BYMV, other potyviruses have sometimes been found in cultivated Crocus, particularly Turnip mosaic virus (TuMV), Iris severe mosaic virus (ISMV) and Iris mild mosaic virus (IMMV) (Novalinskiene and Samuitiene, 2001; Miglino et al., 2005). However, in these cases the examined plants showed symptoms of mosaic and chlorosis of the leaves and petal colour breaking (Table 2). As outlined in Table 2, potyvirus infected plants have been found worldwide, though in some cases these plants were originally derived from corms imported from North Europe. The summary of reports of viruses in Crocus spp. (Table 2) also indicates that two soilborne viruses, Tobacco rattle virus (TRV), which is transmitted by nematodes (Hull, 2002), and the almost ubiquitous Tobacco necrosis virus (TNV), transmitted by the fungus Olpidium brassicae (Hull, 2002) have also been found in these plants. A third virus sometimes found in Crocus spp., is the aphid transmitted Cucumber mosaic virus (CMV) (Bellardi and Pisi, 1987; Samuitiene and Novalinskiene, 2008), possibly the most successful plant pathogenic virus with the widest host range (more than 1,000 plant species, in 365 genera from 85 families) and with worldwide distribution (Gallitelli, 2000). In any case, it should be noted that all the above viruses have been identified in plants with symptoms (Table 2), thus it is not known whether they may be responsible for latent infections as well.

In view of the possible widespread occurrence of potyviruses causing latent infections in Crocus spp. a question to be addressed is the influence of single or multiple viral infections on the productivity and quality of saffron. This is important, considering the positive economic impact that may result from even modest improvements in the production and the profile of important secondary metabolites from virus-free plants. Though no data are available for saffron, it was shown that latent virus infections significantly decrease crop production (Hull, 2002). It is also possible that yield losses often experienced by saffron growers may be in part attributed to latent virus infections, besides other pathogens such as bacteria and fungi listed as main cause of saffron diseases in Italy (Cappelli, 2006) and other parts of the world (Kafi et al., 2006). Certainly, the difficulty in detecting virus symptoms such as colour breaking in Cro-
Crocus fields, and the fact that saffron is considered a minor crop, at least in Europe, possibly account for the few reports on presence and distribution of viruses in these species in scientific literature. Another reason for the scanty reports probably is the fact that in the last 30 years saffron cultivation area has changed dramatically, decreasing to less than 2000 ha in European countries (Greece, Spain and Italy) and increasing to over 50,000 ha in East Asia (mainly Iran and India) (Fernandez, 2004; Gresta et al., 2008). This is due to the lower manual labour costs in these countries. In these new areas of production phytosanitary controls are possibly not carried out.

The European and Mediterranean Plant Protection Organization (EPPO) has published “Guidelines for inspection of Crocus for symptoms of viruses and nematodes”, which state that BYMV symptoms can be best detected after flowering and tend to disappear at the end of the growing season (EPPO Bulletin, 2002). This possible symptom disappearance, and that growers, after flower harvest, usually reduce field inspections, would further explain the paucity of reports about virus infections in the crops. Indeed, until now published papers on saffron almost exclusively outline cultural practices to improve production (reviewed in Gresta et al., 2008), and enhance the biosynthetic pathways involved in the synthesis of the secondary metabolites of major interest (crocetin, picrocrocin and safranal; Bouvier et al., 2003; Carmona et al., 2007; Rubio Moraga et al., 2009) and their natural properties.

Saffron has been considered for many years as a secondary crop, relegated to poor soils and adverse climate conditions, thus not deserving particular attention from the scientific viewpoint (Negbi, 2009). Nevertheless, the current rapid expansion of interest in nutraceuticals, and the discovery or confirmation of interesting properties of saffron, in particular as a cancer chemopreventive and curative (Abdullaev, 2004; Aung et al., 2007), an antidepressant (Wang et al., 2010), an anti-myloegenic in Alzheimer’s disease (Papandreou et al., 2006) and an inhibitor of age-related macular degeneration (Falsini et al., 2010), should increase plant scientists’ interest in this species. In particular, an important goal is the improvement both of quantity and quality of the production, although saffron sterility hampers conventional plant breeding approaches to this target. Phytosanitary selection of virus-free corms, besides the cultural practices, could be of great help to improve production, as it has been for other Iridaceae such as iris and gladiolus, infected by the same set of viruses which affect saffron (Van der Vlugt, 1994; Katoch et al., 2003). Though potyvirus detection in corms is generally more difficult that in leaves, at least in gladiolus and with the ELISA technique (reviewed in Katoch et al., 2003), the multiplex RT-PCR approach has proved to be successful to concurrently detect up to four potyviruses in calla lily plants (Hu et al., 2010), a species which is also affected by latent potyvirus infection (Chen et al., 2004).

Sanitation procedures for saffron corms should also be investigated. Up to now, the only sanitation practices suggested have been for fungal pathogens, and have included fungicide treatments before planting (Tammaro, 1999; Capelli, 2006; Akbari et al., 2010). However, there is the possibility that thermotherapy could be used for eradicating viruses as for other bulbous plants (Conci and Nome, 1991). At this regard Chen et al. (2006) reported successful eradication of TuMV, CMV and TRV from callus and differentiating shoots of C. sativus.

Concluding remarks and perspectives

The presence of latent potyvirus infections need to be investigated in large scale phytosanitary programs, being as these viruses are possibly responsible for the low productivity and decay of many saffron crops worldwide. Unfortunately the previously mentioned EPPO certification scheme for the production of virus free saffron mother stocks (EPPO Bulletin, 2002) includes only visual inspection for symptoms, which obviously will not detect potyvirus latent infections. Thus, this protocol should be implemented, adding at least ELISA tests for the detection of BYMV. Such tests should also be performed by growers’ organizations in each cultivation region, as growers tend to plant their own propagated corms, both for economic reasons and for the rules imposed by the Protected Denomination of Origin (DOP) certification. This protects and safeguards the provenance and origin of product in Europe. In this way, latent virus infections will be maintained indefinitely, unless these latent infections
do not degenerated into more severe infections causing visible symptoms. There is also the possibility that the presence of these viruses contributes to the sterility of saffron and to the quality of the product, which is fundamental in order to obtain a constant set and quantity of secondary metabolites required by nutraceutical and pharmacological applications. The knowledge acquired on these topics is particularly important in Europe where high crop performance, both in terms of quantity and quality, is necessary to for growers to stay competitive in a global market. Furthermore, the possibility of significantly ameliorating crop performance would persuade farmers to cultivate saffron in low-fertility marginal areas where adverse climate prevails and soils are poor, and where saffron can be easily adapted (Negbi, 1999). This expanded cultivation of a valuable crop would improve income and benefit farmers in low income regions.

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