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Genomics and Transcriptomics of Saffron: New Tools to Unravel the Secrets of an Attractive Spice

Alessia Fiore[1*] • Daniele Pizzichini[1] • Gianfranco Diretto[1] • Federico Scossa[1] • Laura Spanò[2]

[1] ENEA C.R. CASACCIA Via Anguillarese 301, 00123 Rome, Italy
[2] Università degli Studi dell'Aquila, Dipartimento di Biologia di Base e Applicata, Via Vetoio 67100, COPPITO L'Aquila, Italy
Corresponding author: * **alessia.fiore@enea.it**

ABSTRACT

Saffron (*Crocus sativus* L.) is a triploid, sterile plant belonging to the *Iridaceae* family and it has been used as a spice and medicinal plant in the Mediterranean area for thousands of years. Saffron is currently considered the most expensive spice available on the global market. Nowadays, an in-depth knowledge of the genomic and transcriptomic organization of saffron represents the main step to fully elucidate the origins of *C. sativus* and the genetic basis of its organoleptic properties. A combination of EST sequencing, characterization of genetic polymorphisms, and "*omics* "approaches will be discussed as effective tools in saffron investigation.

Keywords: apocarotenoids, CCD, *Crocus sativus*

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INTRODUCTION

Saffron is the name given to the desiccated stigmas of *Crocus sativus* L., a triploid, sterile plant, propagated by corms, belonging to the family *Iridaceae* (Deo 2003). The word "saffron" comes from the Arabic: "za-faran" (yellow) and the powder obtained from the dried stigmas is renowned today as the world's most expensive spice.

Main properties of saffron are its bright orange-red colour, the bitter taste, flavour and aromas that confer to many traditional and seafood meals, especially in Asian and western European countries. In ancient times, frescoes paintings from Knossos, Crete, (dated back to 1700 B.C.) witness the use of saffron in the Minoan civilization (Deo 2003). In fact, *C. sativus* was probably domesticated by Minoan farmers between 3000 and 1600 B.C., when *Crocus* plants were selected on the basis of their pigmented stigmas. Saffron was also consumed by Romans and Greeks, who believed in its aphrodisiac properties and was used both as spice for food and wines, as a dye and in the preparation of several perfumes (Hill 2004). From the Middle Ages to the industrial revolution, the diffusion of saffron was accompanied by a constant increase in its commercial value. Once perceived as a "luxury" spice, saffron began to be adulterated with low-quality colouring agents and inorganic ingredients. In recent years, a novel interest has emerged with respect to the pharmacological properties and antioxidant potential derived from the consumption of saffron in human diet.

The red stigmas of *Crocus* accumulate three different apocarotenoids (i.e. products derived by the enzymatic cleavage of a carotenoid precursor): crocin, picrocrocin, and safranal (**Fig. 1**), which are responsible, respectively, for the colour, taste and aroma of saffron (Kanakis *et al.* 2004). The ability to synthesize these compounds is not common across species: picrocrocin and crocin, in fact, have only been identified in stigma tissues of some *Crocus* species and few others species such as *Buddleja* (Liao *et al.* 1999) and *Gardenia* (Pfister *et al.* 1996). Because of the predominant accumulation of apocarotenoids in the stigmas of *C. sativus*, and due to their potential antioxidant effects, their biosynthesis has been extensively investigated.

BOTANY OF *CROCUS SATIVUS*

C. sativus shows perennial, herbaceous, rosette growth and has permanent underground stem bases, called bulb or corm, almost spherical with a 3-5 cm diameter (Hill 2004; Molina *et al.* 2004). *C. sativus* is an autumn flowering geophyte with subhysteranthous behaviour and leaf emergence coincides or occurs shortly after flowering, withering at the onset of the dry season, while during late spring and most of summer plants show no aboveground organs or roots (condition usually called "dormancy"). Flower initiation usually occurs during this period and its formation is usually restricted to the apical and dominant bud in non-flowering shoot-derived corms, while it can occur in two or three apical buds in flowering shoot-derived corms (Molina *et al.* 2004). Saffron growth cycle usually lasts around 220 days and favourable climatic conditions for high yields of

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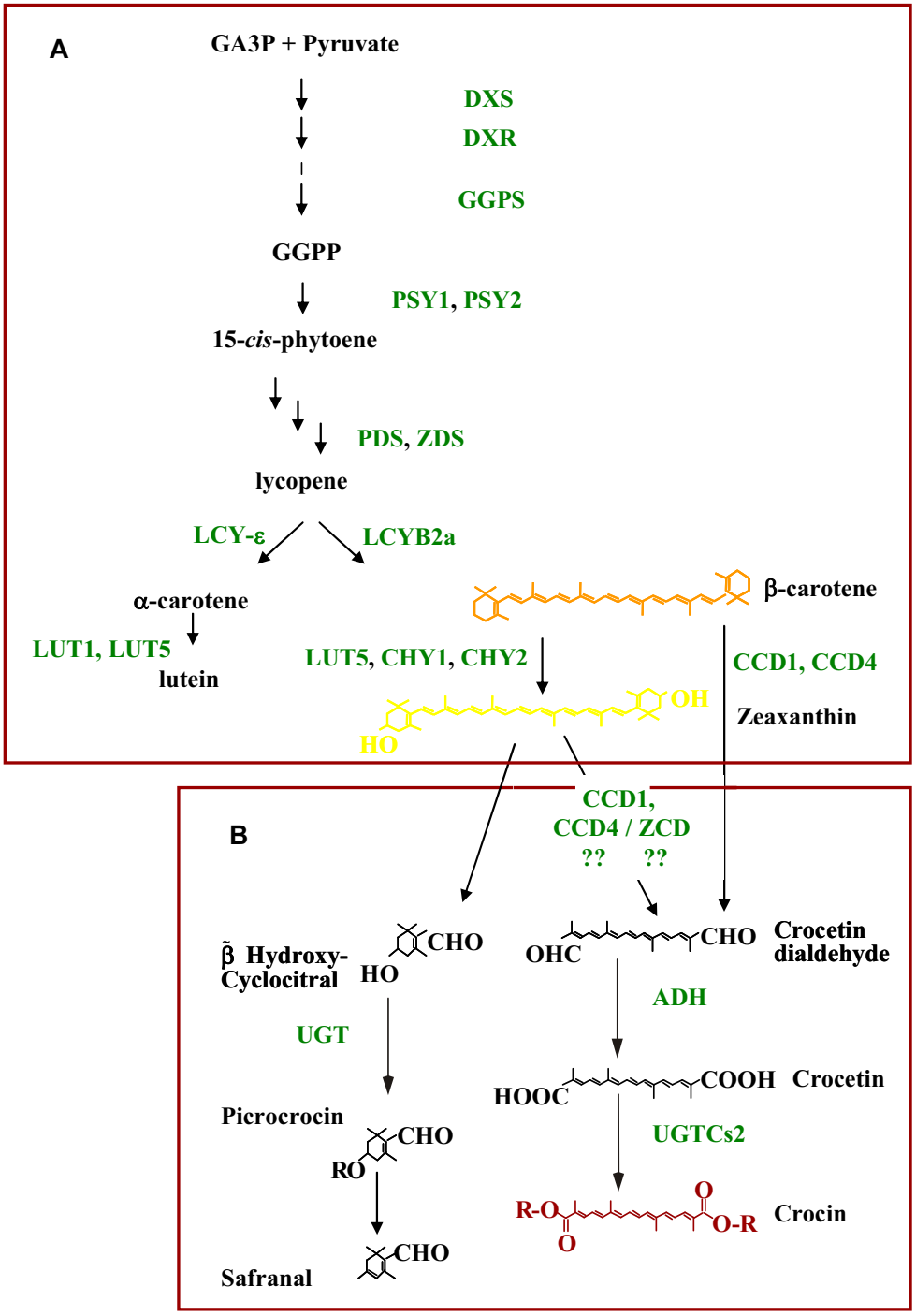

Fig. 1 Biosynthetic pathway of three major carotenoid derivatives, crocetin, picrocrocin, and safranal in *C. sativus* stigma. (**A**) Carotenoid biosynthetic pathway until zeaxanthin (Diretto *et al.* 2007). GA3P, glyceraldehide 3-phosphate; DXS, 1-deoxyxylulose 5-phosphate (DOXP) syntase; DXR, DOXP reductoisomerase; GGPP, geranyl geranyl diphosphate; GGPS, geranyl geranyl diphosphate synthase; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CHY, β-carotene hydroxylase; LUT1, ε-carotene hydroxylase; LUT5, ε–β carotene hydroxylase; LCY-ε, lycopene ε-cyclase LCYB2a, lycopene β cyclase isolated in *C. sativus* (Ahrazem *et al.* 2009); (**B**) Biosynthetic pathway of saffron apocarotenoids (according to Bouvier *et al*. 2003; Moraga *et al*. 2008). CCD, carotenoid cleavage dioxygenase; ADH, Aldehyde dehydrogenase; UGT, UDPG-Glucosyltransferases; UGTCs2, UDPG-Glucosyltransferases isolated in *C. sativus* (Moraga *et al.* 2004); ZCD, zeaxanthin cleavage dioxygenase (Bouvier *et al.* 2003).

saffron are rainfall in the autumn, warm summers and mild winters. Water requirements are low and garden (clay sand) soils allow the optimum growth. These features, together with the very low harvest make saffron a highly remarkable agrological and eco-physiological species.

GEOGRAPHICAL DISTRIBUTION OF *CROCUS SATIVUS* AND SAFFRON PROPERTIES

Saffron is considered the highest priced spice in the world (on average, 500 $/kg) (Hill 2004). Its high price is due to the direct manual labour required for its cultivation, harvesting and handling. One stigma of saffron weighs about 2 mg, each flower has three stigmata, and 150,000 flowers must be carefully picked in order to produce 1 kg of spice.

Virtually, saffron is produced in a wide geographical belt extending from the Mediterranean area in the west to the Kashmir region in the east. All the continents outside of this zone, except for Antarctica, produce smaller amounts. Annual worldwide production amounts to around 205 tonnes (Schmidt *et al.* 2007), including whole threads and powder. Iran, Spain, India, Greece, Azerbaijan, Morocco, and Italy (in decreasing order of production) dominate the world saffron market, with Iran and Spain accounting for

80% of the total. Despite numerous cultivation efforts in countries especially in Austria, United Kingdom, Germany, and Switzerland, only few locales cultivate saffron in Northern and Central Europe.

Saffron has a sweetish aromatic odour and a bitter taste. It is mainly used as spice or condiment, adding its faint, delicate aroma, pleasing flavour and magnificent yellow colour 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, mustard, chocolate and liqueurs (Basker *et al.* 1983). The commercial quality of saffron depends heavily on its colouring strength, bitterness and aroma intensity. Saffron is also considered a highly valued medicinal plant and many pharmaceutical uses have been reported so far (Schmidt *et al.* 2007). Traditionally, it has been used against cramps, bronchospasms, liver and menstruation disorders (Abdullaev 2003). Very important applications are also in supportive treatments of various forms of cancers (Dhar *et al*. 2009), in anti-inflammatory responses (Hosseinzadeh *et al*. 2002) and in anti-depressive therapies (Hosseinzadeh *et al*. 2004).

APOCAROTENOID BIOSYNTHESIS AND MOLECULAR INVESTIGATION IN SAFFRON

Plant apocarotenoid biosynthesis starts with geranyl diphosphate that is deemed to be the universal precursor for monoterpenes, key constituents of flower, fruit, and spice plant aromas (Croteau *et al.* 2000). Geranyl diphosphate is converted into geraniol by the activity of geraniol synthase (direct pathway). In several plants, geraniol is readily oxidized to geranial by alcohol dehydrogenases but, anyway, it has also been shown that geranial and other apocarotenoids can be formed *in vitro* by oxidative cleavage of lycopene.

The synthesis can also proceed indirectly, through the oxidative cleavage of carotenoids: farnesyl acetone and geranyl acetone are produced from phytoene; pseudoionone, neral and geranial are instead obtained from the cleavage of neurosporene, pro-lycopene and lycopene; finally, β-carotene is the precursor of a third group of volatiles including β-cyclocitral, β-ionone and dihydroactinodiolide. Citral, a mixture of *cis* and *trans* noncyclic monoterpene aldehyde isomers (neral and geranial, respectively) possesses an agreeable scent, reminiscent of lemon (Lewinsohn *et al.* 2005) and it is a major component of lemon basil (*Ocimum basilicum* L., Lamiaceae), lemongrass (*Cymbopogon citratus*, Poaceae), litsea (*Litsea cubeba* Pers., Lauraceae), and other lemon-scented aromatic plants. Citral has also a major impact in the aroma of tomato and watermelon (*Citrullus lanatus*), two fruits accumulating high levels of the tetraterpene red pigment lycopene (Lewinsohn *et al.* 2005).

It is presently unknown whether citral accumulating in lycopene-rich fruits is directly derived from geranyl diphosphate or if it is produced by oxidative degradation of lycopene. Wild-type tomatoes also accumulate specific noncyclic volatile norisoprenoids, such as 6-methyl-5-hepten-2-one, farnesyl acetone, (*E*,*E*)-pseudoionone, 2,3-epoxygeranial, 2,6-dimethylhept-5-1-al, geranyl acetone, and dihydro-*apo*-farnesal, as well as the monoterpene aldehydes geranial and neral (citral) and the cyclic norisoprenoid α-ionone (Lewinsohn *et al.* 2005).

Crocus flowers show red style branches, which, upon desiccation and once reduced to a powder, constitute the spice saffron. It has been proposed that the biogenesis of the three major carotenoid derivatives, crocetin glycosides (crocins), picrocrocin, and safranal, which are responsible for saffron colour, bitter taste and aroma, is derived from the oxidative cleavage of the carotenoid zeaxanthin (Bouvier *et al.* 2003) (**Fig. 1**). This step leads to the formation of a polyene molecule (crocetin dialdehyde) and two identical β-ionone molecules, hydroxyl-β-cyclocytral. The oxidated form of crocetin dialdehyde (crocetin) constitutes the substrate of another reaction probably catalyzed by an UDP-glucosyltransferase, with the formation of glucosyl esters of crocetin (named crocins, which can be further classified in different subtypes on the basis of the transferred sugar moiety). On the other side of the pathway, the β-ionone hydroxyl-β-cyclocytral, derived from the initial cleavage of zeaxanthin, is thought to be converted by an UDP-glucosyltransferase to picrocrocin and safranal (Moraga *et al.* 2004). The first genes that were cloned, identified and functionally characterized in *Crocus* are *CsZCD* (zeaxanthin cleavage dioxygenase) and *CsCCD* (carotenoid (9', 10')-cleavage dioxygenase) (Bouvier *et al.* 2003). The expression of *CsZCD* seems to be restricted to the style branch tissues and it is enhanced under dehydration stress, whereas *CsCCD* is expressed in a constitutive manner in flower and leaf tissues and irrespectively of dehydration stress. Bouvier and co-authors (Bouvier *et al.* 2003) suggested the existence of a stepwise sequence involving the oxidative cleavage of zeaxanthin inside the chromoplasts followed by the sequestration of modified water-soluble derivatives into the central vacuole.

The subsequent step involves glucosylation of crocetin and β-hydroxy-ciclocytral, with the formation of, respectively, crocin and picrocrocin. These glucosylation reactions are catalysed by various glucosyltransferases (GTases), belonging to a class of enzymes involved in the biosynthesis of several plant secondary metabolites (glycoalkaloids, anthocyanins, betalains, etc.). The Gómez-Gómez group cloned and studied the expression of a gene coding for a saffron glucosyltransferase able to glucosylate crocetin (Moraga *et al.* 2004). Glucosylation of crocetin is very important since it confers stability and water solubility to the pigment and improves its bioavailability and, thus, its pharmaceutical interest. Additional genes involved in saffron carotenoid biosynthesis have been characterized so far: partial clones encoding phytoene desaturase, phytoene synthase and carotenoid dioxygenases from stigma tissues have been isolated, and their expression has been analyzed during stigma development and carotenoid accumulation. High expression level of carotenoid dioxygenase 3 clone (*CsCCD3*) suggests that the gene product could be involved in saffron apocarotenoid biosynthesis (Rubio *et al.* 2004).

Castillo and co-workers observed that expression level of *CsBCH* (β-ring hydroxylase) is dependent from the relative levels of zeaxanthin in the stigma, suggesting that activity of this enzyme could represent a limiting step in the apocarotenoid formation (Castillo *et al.* 2005).

More recently, four additional genes encoding carotenoid cleavage dioxygenase have been isolated from *C. sativus*: *CsCCD1a*, *CsCCD1b*, *CsCCD4a* and *CsCCD4b* which show a very variable pattern of expression among different tissues. *CsCCD1b* was expressed only in stigma tissues and the expression levels of both *CsCCD4a-4b* correlated with the accumulation of β-ionone during stigma development (Rubio *et al.* 2008). Moreover, bioinformatic analyses have showed that the deduced amino acid sequences of several carotenoid dioxygenases from a variety of plant organisms cluster into four distinct subfamilies: CCD1, CCD4, NCED and a fourth class including both CCD7 and CCD8. It has been shown that CCD1 family possesses cleavage activity on a variety of carotenoid substrates, while members of CCD4 family would only be able to cleave β-carotene. Rubio and co-workers also assessed that, in their experimental conditions, CsZCD enzyme lacks of cleavage activity; expression of *CsZCD* gene in zeaxanthin-accumulating *E. coli* strain resulted in, unexpectedly, no cleavage activity. CsZCD enzyme would, thus, represent a truncated N-terminal form with respect to the CCD4 protein, lacking of plastid target sequence. On the basis of the discordant data between the two groups (Rubio and co-workers, Bouvier and co-workers), CsZCD enzyme characterization and its catalytic activity would need a more detailed and accurate re-evaluation.

Recently, studies of a number of saffron enzymes involved in flavonoid glucosylation (flavonoid glucosyltransferares, Rubio-Moraga *et al.* 2009) and carotenoid biosynthesis (lycopene β-cyclase, Ahrazem *et al.* 2009) have been

performed, increasing the number of candidate genes which are responsible for the high-valuable saffron organoleptic features.

GENOMIC ORGANIZATION IN *CROCUS SATIVUS*

A large genome size (around 30,000 Mbp and, thus, twice, 60 and 240 times larger than, respectively, *Triticum estivum*, *Oriza sativa* and *Arabidopsis thaliana*) has been estimated for *C. sativus* on the basis of the size of the diploid specie *C. vernus* (11,000 Mbp; Chichiriccò 1984).

Saffron is a triploid specie with basic chromosome number of x=8 (Chichiriccò 1984). The karyotype of *C. sativus* has been studied by several authors and on different ecotypes from several countries (Azerbaijan, Iran, Italy, Turkey, France and United Kingdom) and it is always resulted as 2n=3x=24, without any significant karyologycal difference. The accepted karyotype is, then, composed of 8 triplets: subacrocentric (1, 2), metacentric (3, 4 and 8) and submetacentric (6 and 7) chromosomes. Three chromosomes in each triplet, as a rule, are similar although in some triplets one of them is infrequently distinguishable from the others. Triplet 5 shows a marked as it contains two distinct chromosomes subtypes: chromosome 5(1), metacentric, and chromosome 5(2,3), subacrocentric and smaller.

Using a combination of genetic, molecular and cytological methods, genomes of several *Crocus* species have been investigated: Heslop-Harrison and co-workers (Frello *et al.* 2000), for example, through a recombinant DNA library, isolated eight clones of repetitive DNA in *C. vernus* Hill. The DNA organization was analyzed by *in situ* hybridization and Southern analyses in a broad range of *Crocus* species. Sequence analysis evidenced that all 8 clones were non homologous, and suggesting, thus, the presence of 8 different sequence-families. Almost all clones, analyzed by *in situ* hybridization, displayed a dispersed organization at high copy numbers on all chromosomes of the *C. vernus* genome. In only one case, a specific sequence has been found showing high homology to the reverse transcriptase gene of Ty1-*copia*-like retrotransposons. The genomic distribution of clones seemed to be discordant with the taxonomy classification, therefore this data suggested that a more detailed analysis of phylogeny and taxonomic structure should be done. Moreover, the genomic distribution and organization of two clones of highly repetitive DNA previously described (Frello *et al.* 2000), were further studied (Frello *et al.* 2004). The sequences of the two clones were 85% identical; the presence of these sequences was monitored in 54 *Crocus* taxa and in some species of other genera. These findings suggested that both sequences were specific to *Crocus*. Unfortunately, the distribution of hybridization signal across the genus showed poor agreement with the taxonomic structure, confirming that taxonomy of the *Crocus* genus might need additional and novel re-evaluation.

More recently, many efforts have been performed to achieve a better understanding of the genomic organization of *Crocus* species. Seberg *et al.* (2009) presented the analysis of a proposed barcode set of genes (Chase *et al.* 2007) in the genus *Crocus*. *RpoC1*, *matK* and *tmH-psbA* regions were analyzed on 86 species of the *Crocus* genus and the proposing sets were further extended with several other genomic regions to obtain a final diagnostic set for 79 out of the 86 analyzed species. The authors asserted that, although barcoding is still unable to identify more than 75% of the known species, it represents a very promising and powerful system and more efforts in this technology are thus envisaged in the near future.

In a more recent paper, Moraga *et al.* (2009) analyzed the RAPD profile of 43 isolates of *C. sativus* to determine the morphism of this species. Using three different approaches (random amplified polimorphic DNA, intersimple sequence repeats (ISSR) and microsatellite analysis), they assessed the variability of saffron from several different geographic areas and concluding that *C. sativus* is a monomorphic species.

A new genome walking approach named "rolling circle amplification of genomic templates for inverse PCR" (RCA-GIP) has been proposed (Tsaftaris *et al.* 2009). This method consists of a rolling circle amplification of the circular DNA generated from restriction analysis of genomic DNA by using different enzymes and through a subsequent ligation with appropriate adaptors. Using this method, four promoter regions of flowering genes (Tsaftaris *et al*. 2007) from *C. sativus* were isolated. The promoter analysis showed a presence of common promoter elements as the TATA box and binding motifs for transcriptional factors. The RCA-GIP method allowed the isolation of genomic sequence flanking several genes; this is an important step to understanding the complicated mechanisms of gene regulation.

TRANSCRIPTIONAL ANALYSIS OF *CROCUS SATIVUS*

The characterization of the saffron transcriptome is a very important starting point to shed light on several high-valuable biological processes such as the molecular basis of flavour and colour biogenesis and the genomic organization of *Iridaceae*. The group of the authors presenting this review carried out a large-scale study dealing with the construction of an Expressed Sequence Tags (EST) database from saffron stigmas. In this work (D'Agostino *et al.* 2007), espression profiling of mature stigmas was evaluated by means of collection and sequencing of an EST pool. Bioinformatic analysis of the entire dataset provided a first general overview of the transcriptome organization of this species with a particular emphasis on the organ involved in saffron spice production.

The global sequencing of a cDNA library produced from mature stigmas of *C. sativus* coupled to extensive and bioinformatic analyses of sequence data allowed the construction of the first saffron database (www.saffrongenes.org). "Saffrongenes" is a freely available resource for the research community involved in saffron genomics (D'Agostino *et al.* 2007).

Several bioinformatic analyses were performed; 7965 ESTs were obtained through remotion of the vector regions and were subsequently subjected to a batch analysis (BlastX) in order to identify significant homologies. Within the pool of EST selected (7965), 5355 EST (67%) give no significant homologies and 2610 (33%) ESTs showed significant homologies. The 2610 ESTs were further separated in non-redundant (the typologies of homology, NR-set) and redundant (EST belonging to each classes, R-set) sets. NR-set (392 elements) accounts the independent classes of homology excluding their occurrence (number of EST belonging to a specific class), whereas redundant R-set includes the homology classes and the number of EST in each classes (2610 elements). A manual classification in 10 functional classes was performed (**Fig. 2**) for both datasets in order to identify most representative classes: Regulation (19% NR-set, 16% R-set), metabolism (19% NR-set, 17% R-set), stress response (16% NR-set, 19% R-set).

A further classification and more accurate investigation were conducted on the EST initial pool using a dedicated platform of bioinformatic clustering, assembly and annotation of EST, named ParPEST (D'Agostino *et al.* 2005). The analyzed pool comprises 6,603 high quality ESTs from a saffron mature stigma cDNA library. The ESTs have been grouped into 1,893 clusters, each corresponding to a different expressed gene, and subjected to a subsequent annotation. Evaluation of homologies after BlastX analysis evidenced the high expression level of some transcript contigs (TCs). In the context of ESTs analysis, the number of ESTs members in each unique gene is an indication of the expression level of that gene. The most represented homologies in *C. sativus* stigmas were grouped in three different ontology classes: molecular function, biological process and cellular component. Catalytic activity and transport were the most represented category in, res-

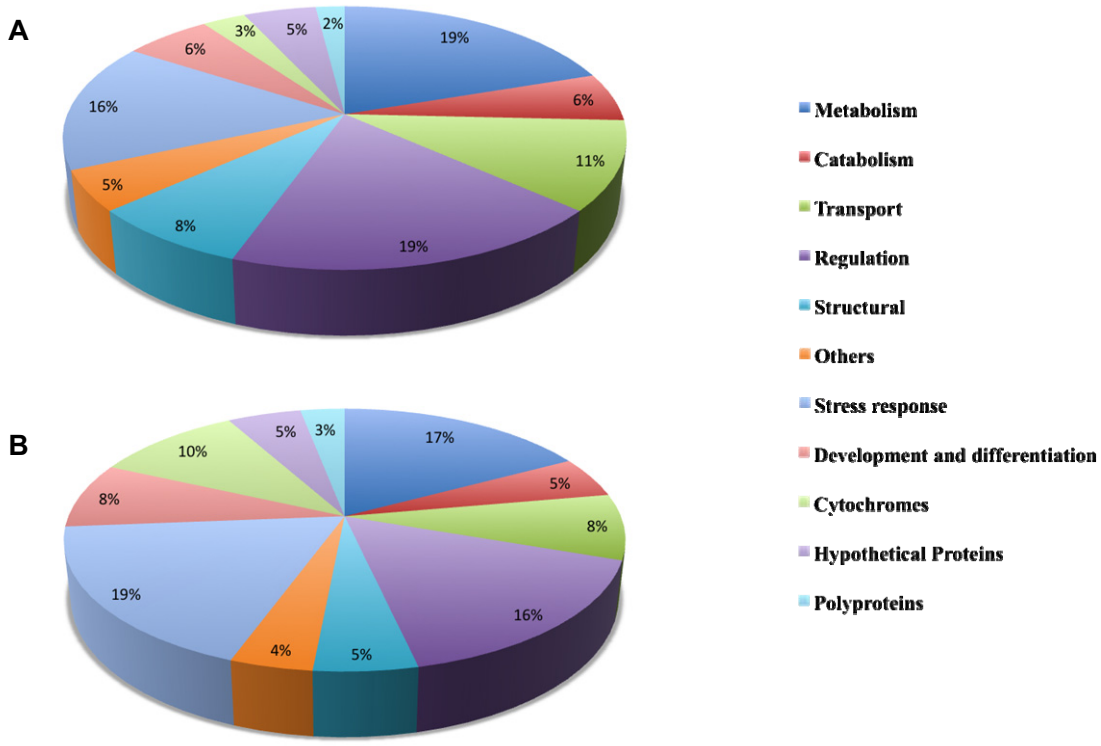

Fig. 2 Functional classification of ESTs: (**A**) non redundant set, (**B**) redundant set.

pectively, molecular function and biological process classes, whereas a more uniform distribution was observed in the cellular component class, with similar levels among the three cellular compartments: plastid, mitochondrion and cytoplasmic membranes. The most highly expressed TC, Cl000057:2 (547 ESTs), revealed homology to short chain dehydrogenases and is homologue to *TASSELSEED2 (TS2)* of maize that is involved in sex organ differentiation and in the genetically determination of programmed pistill cell death (Calderon-Urrea *et al.* 1999). Moreover, many P450 (122 ESTs) and b5 (45 ESTs) cytochromes were recovered from the sequencing of saffron stigmas libraries; the high abundance of these transcripts may be related to the primary role that cytochromes have in stigma metabolism and development. Several ESTs clustered into TCs related to enzymatic activities which are specifically involved in secondary metabolisms (carboxyl methyltransferase (42 ESTs) and UDP-gycosyltransferase (33 ESTs)) while some other TCs, encoding putative proteins with high homology to heat shock proteins of *Arabidopsis thaliana* (TC, Cl001114:3, 104 ESTs), lipid transfer proteins (94 ESTs) and to transcription factors such as the Myb-like factors (54 ESTs) and MADS box (36 ESTs) were also identified. The Saffrongene database (www.saffrongenes.org) has been designed to manage and to explore the EST collection from saffron stigmas, providing a reference for the expression pattern analysis in this tissue as well as a primary view of the genomic properties of this species, and the database represents the first reference collection for the genomics of *Iridaceae*, for the molecular biology of stigma biogenesis, as well as for the metabolic pathways underlying saffron secondary metabolism.

A "local" transcriptomic approach has been also performed to study the expression of specific classes of genes: Tsaftaris and collaborators provided, for example, a detailed investigation of regulatory genes involved in flowering time and flower development (Tsaftaris *et al.* 2009). In this study, a combination of conventional (5'- and 3'-RACE PCR) and new methods (Rolling Circle Amplification RACE, and familyRCA-RACE) has been employed to further characterize the structure and expression of specific gene families. Several full-length cDNA clones encoding MADS-box transcription factor proteins involved in flower formation were cloned and characterized; within this group, five PISTILLATA/GLOBOSA-like (PI/GLO-like) MADS-box genes were isolated and gene expression studies detected their expression, in the outer whorl tepals which are the sepals in most typical flowers (Kalivas *et al.* 2007).

In another study, the Gómez-Gómez group (Rubio Moraga *et al.* 2009) used a combination of approaches to study the accumulation of colour and aroma compounds during stigma development performing an *in silico* screening of the stigma cDNA database previously described (D'Agostino *et al.* 2007) and a GC-MS profiling of stigma metabolites, in order to identify candidate genes encoding enzymes involved in saffron volatile biosynthesis. In this way, authors identified several genes showing highly increased expression during stigma development: this group comprises two putative terpene synthases, TS1 and TS2 and two carotenoid gene transcripts, *CsPSY* (phytoene synthase) and *CsPDS* (phytoene desaturase). Taken together, these data suggest expression pattern of several candidate genes is strongly correlated with volatile production and organoleptic characteristics during stigma development.

In any case, a detailed large-scale analysis of the transcriptome and metabolome of *Crocus species* should provide additional details on early developmental stages of saffron stigmas, where the transcription of genes involved in flavour and aroma production leads to the accumulation of secondary metabolites.

CONCLUSIONS

In recent years, a combination of different approaches has been employed in *Crocus* species to better characterize the available genetic resources, as well as the organization and composition of its genome and the transcriptional activity of saffron-accumulating tissues. Several genes responsible for flavour, aroma production, carotenoid and flavonoid accumulation have been isolated and functionally characterized. However, the metabolism of the stigmas of *Crocus* is still poorly characterized, despite the recent progresses made in elucidating the biosynthesis and accumulation of the major apocarotenoids. A more extensive investigation of *Crocus* metabolites involved in primary/secondary metabolism, which accumulate during stigma development, is thus

needed, with a particular emphasis on those volatile compounds which are presumably involved in the generation of the saffron aroma. In the near future, the rapid evolution of next-generation DNA sequencing technologies and the recent progresses at the level of sample throughput and instrumental resolution of analytical platforms for metabolite structural characterization (e.g., high-resolution mass spectrometers) will offer a promising, combined approach to correlate the genetic determinants with the corresponding biochemical phenotypes. The detailed elucidation of the genetic and biochemical basis responsible for saffron organoleptic qualities and pharmaceutical properties will thus require an integration of different high-throughput "*-omics"* techniques.

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