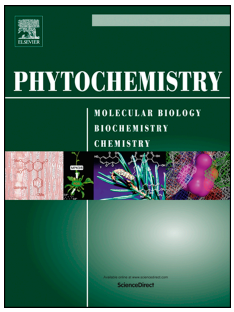

An integrated approach for the characterization of wild *Crocus* species adopting phenotypical and phytochemical traits

Laura Siracusa, Andrea Onofri, Rosario Galesi, Carmen Impelluso, Luana Pulvirenti, Giuseppe Ruberto, Fabio Gresta, Giovanni Spampinato, Antonia Cristaudo

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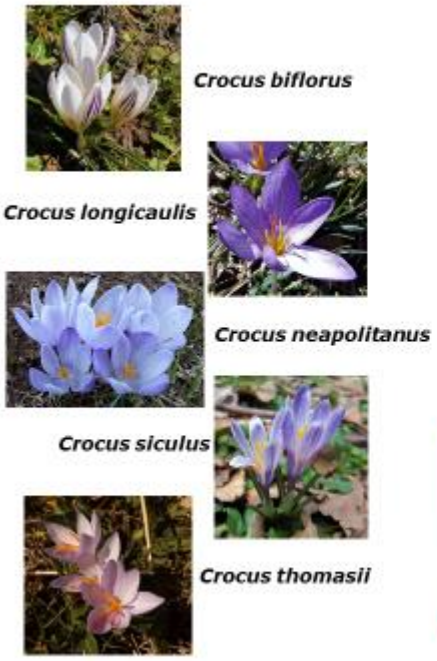
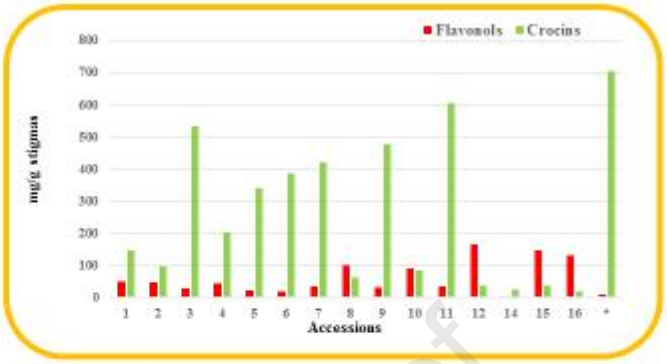
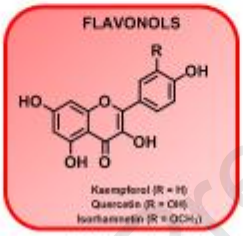
Graphical Abstract

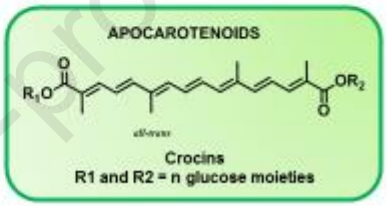
Sixteen samples of wild *Crocus* belonging to five species collected in southern Italy have been studied. Morphological traits and content of carotenoids and flavonoids have been evaluated and compared.





Flavonols and crocins content of 15 accessions of Crocus (*C. *sativus*/saffron)





An integrated approach for the characterization of wild *Crocus* species adopting phenotypical and phytochemical traits

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ABSTRACT

Crocus is a taxonomically complex genus, characterized by high intra- and inter-specific variability. Five wild *Crocus* taxa - *Crocus biflorus*, *C. longiflorus*, *C. neapolitanus*, *C. siculus* and *C. thomasii* from three different areas of Southern Italy (Calabria, Basilicata and Sicily) have been investigated. We considered both flower morphological traits (inner and outer perigone segments, style branches and anthers) and the chemical profile of specialized metabolites (apocarotenoids: crocins; flavonoids: flavonols) in style branches, which were determined by high-performance liquid chromatography, coupled with a diode array detector and mass spectrometry (HPLC-UV-DAD-ESI-MS). Saffron (*C. sativus*) was also included as the 'control' species. The length of perigone tube, outer and inner tepals, anthers and style branches gave the highest contribution to the discrimination of different taxa. Concerning the specialized metabolite profiles, 20 flavonols and 24 crocins have been identified and quantified in the *Crocus* extracts and used to discriminate among samples, confirming that *Crocus* taxa can be considered as an important natural source of these substances. The chemical profiles of the different populations showed some distinctive qualitative and quantitative patterns that contributed to a certain degree of discrimination among species, in fact, flavonoids content range is comprised between 2.7 and 145.9 mg/g, whereas crocins range between 19.8 and 604.0 mg/g. It is thus hypothesized that the combination of morphological and phytochemical screenings may be an effective methodology to characterize wild *Crocus* genotypes from Southern Italy, also in comparison to *C. sativus* (saffron).

Keywords: *Crocus biflorus*; *C. longiflorus*; *C. neapolitanus*; *C. siculus*; *C. thomasii*; Iridaceae; morphological characterization; chemical characterization; crocins; flavonols.

Abbreviations

Gas chromatography: GC

Gas Chromatography-Mass Spectrometry: GC-MS

High Performance Liquid Chromatography: HPLC

High Performance Liquid Chromatography-Diode-Array Detector: HPLC-DAD

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry: LC-ESI-MS

Nuclear Magnetic Resonance: NMR

Restricted Maximum Likelihood: REML

Ultraviolet-Visible: UV-vis

1. Introduction

The genus *Crocus* L. belongs to the large family of Iridaceae and consists of about 90 perennial geophytes taxa (Mathew,1982; Petersen et al. 2008), although recent studies suggest around 150-200 species (Harpke et al. 2013, 2015, Carta et al. 2015; Peruzzi, 2016; Roma-Marzio et al., 2018). These species range from Central Europe to North Africa and from Southwest Asia to Western China, with predominant centres of diversity in the Balkan Peninsula and Turkey (Peruzzi, 2016; Gresta et al., 2017; Roma-Marzio et al., 2018).

Plant species of this genus are polymorphic, with complex intraspecific variability. Due to the ambiguity between genetic and morphological traits observed within species, this taxon has been traditionally surrounded by multiple taxonomic controversies (Sheidai et al., 2018). However, significant progresses have been made in recent decades in systematics at the species level, mainly due to the large-scale sampling of plant material and comparative approaches, based on several methods and techniques in concert (e.g. morphometric, chemical, cytotaxonomic, molecular analyses). These data, together with detailed knowledge on distribution patterns, ecological requirements and comparison systems, have favored the delimitation of closely related taxa (Harpke et al., 2013; 2015; Anabat et al., 2022).

The species of this genus occur in environments characterized by cold winter, autumn-winter-spring precipitation, and hot and dry summer. The plants actively grow from autumn to late spring, surviving the summer drought beneath the soil by means of a compact corm (perennial geophytes). They are characterized by a wide heterogeneity in terms of flowering: numerous species bloom after have developed their aerial parts (leaf

79 organs), while some others flower simultaneously with leaf development or soon
80 thereafter. Some species flower in autumn while others flower in spring.

81 Many *Crocus* species are highly prized as garden plants for their colorful flowers,
82 horticultural varieties, for industrial applications and as unique collector's items (Rashed-
83 Mohassel, 2007; Petersen et al., 2008; Fernandez et al., 2011).

84 *Crocus* genus is currently subdivided into two subgenera: *Crocus* and *Crociris*,
85 the first subgenus comprises two sections (*C.* section *Crocus* and *C.* section *Nudiscapus*)
86 and 15 series (Harpke et al., 2016). These sections were defined by some important
87 characters such as bracteoles, division of the style, color of the anthers and flowering
88 time.

89 According to Bartolucci et al. (2018), twenty species of *Crocus* occur in Italy and
90 they are all included in the subgenus *Crocus*. Among these species, in the section *Crocus*
91 series *Verni*, we find the spring-flowering *C. siculus* Tineo ex Guss., which is short-styled
92 and endemic to northern Sicily. Besides, we find the long-styled species *C. neapolitanus*
93 (Ker Gawl.) Loisel., occurring in the Central and Southern Italian peninsula. In the same
94 series, Harpke et al. (2015) also include the autumn flowering species *C. longiflorus* Raf.,
95 that is spread in Southern Italy and Sicily. The *Crocus* series *Sativus* (*C.* section *Crocus*)
96 includes *C. thomasii* Ten. which is widespread in the Adriatic coastal mountains of
97 Western ex Yugoslavia and Southern Italy where it prefers stony slopes or thin scrub,
98 from 0 up to 1000 m asl. This taxon, in addition to eight other species, has been proposed
99 as a potential ancestor of *C. sativus* (Grilli Caiola and Canini, 2010; Nemati et al., 2019).

100 The *Crocus* section *Nudiscapus*, series *Biflori*, characterised by trilobe style and
101 winter flowering (Mathew, 1982), includes *C. biflorus* Mill., an Italian endemism
102 occurring in all regions, except Valle d'Aosta and Sardinia (Harpke et al., 2016;

Bartolucci et al., 2018; Roma-Marzio, 2018). These plants usually show white or lilac flowers with three-to-five striking violet longitudinal stripes on the outer tepals. The colour of the flowers, in particular, represents a peculiar characteristic that has been studied for the entire genus by many authors, especially with reference to the nature and chemical structure of pigments (e.g. anthocyanins and other flavonoids) (Nørbæk et al., 2002). Notwithstanding their valuable chemical constituents, there are very few studies based on morpho-chemical approach on wild *Crocus* taxa.

In this study, we investigated morphological and phytochemical traits of sixteen populations, belonging to five wild *Crocus* species occurring within the Mediterranean floristic region (Southern Italy: Basilicata, Calabria and Sicily), i.e. *Crocus biflorus* Mill., *C. longiflorus* Raf., *C. neapolitanus* (Ker Gawl.) Loisel., *C. siculus* Tineo ex Guss. and *C. thomasii* Ten. (Iridaceae) (Table 1). The aim was to contribute to the characterization of wild *Crocus* taxa, by delimiting species and population boundaries and exploring the relationships between them.

2. Results

2.1. Morphological traits

The analysis of five quantitative morphological traits [length of perigone tube (Lpt), outer perigone segments (Lops), inner perigone segments (Lips), anthers (Lanth) and style branches (Lsb)] revealed very different features among the taxa (Table 2), each of them marked by peculiar character combinations.

C. longiflorus showed the longest perigone tube (83.5 mm), mainly due to the population n. 7, which exhibited the absolute highest value (135.9 mm). *C. thomasii* showed a value of 71.4 mm, while in *C. biflorus* and *C. neapolitanus* the lowest similar

values were recorded the lowest similar values (54.8 and 52.7 mm, respectively). The longest outer perigone segments (tepals) were observed in *C. neapolitanus* (37.6 mm), followed by *C. longiflorus*, *C. thomasii* and *C. biflorus* (33.2, 32.7 and 31.0 mm, respectively) and by *C. siculus* (26.6 mm). A similar pattern was observed for the inner perigone segments: *C. neapolitanus* showed the longest value (34.8 mm), followed by *C. longiflorus*, *C. thomasii* and *C. biflorus* (29.9, 29.0 and 28.4 mm, respectively) and by *C. siculus* (24.1 mm). The longest anthers were measured in *C. thomasii* and *C. neapolitanus* (14.8 and 14.0 mm, respectively). *C. longiflorus* and *C. biflorus* exhibited an intermediate anthers length (12.5 and 10.9 mm), while the shortest anthers were detected in *C. siculus* (8.3 mm). Finally, as far as style branches length *C. longiflorus* clearly emerged as the *Crocus* species with the highest value (13.6 mm), followed by *C. thomasii* and *C. biflorus* (10.0 and 9.1 mm, respectively). The shortest style branches were recorded on *C. neapolitanus* and *C. siculus* (4.7 and 3.9 mm, respectively).

Although the difference between species is rather high for all morphological traits under investigation, the variability between species is almost always lower than the variability between populations, within each species (Table 3). In particular, the variance component for populations (within each species) is lower than the variance component for species only for anther length and style branch length, which supports the idea that not all morphological traits are helpful in discriminating between the different species.

Principal Component Analyses (PCA, Figure 1) shows which taxa are most alike; the resulting biplot explains 85% of the total multivariate variation and shows that the two populations of *C. siculus* are very similar to each other and present low values (below average) for all morphological traits. The two populations of *C. neapolitanus* are also very similar and they are above average for Lops, Lips and Lanth. The other taxa show

wide variability and they are difficult to discriminate based only on morphological traits. Relating to *C. longiflorus*, we observed three putative groups: the first one contains the two populations 7 and 5, which are very similar and characterized by a very long perigone tube, the second group contains populations 4 and 11, which are far below the species average for all traits and the third group, containing all other populations, which are close to average for all traits. The only population of *C. thomasii* appears to be very similar to the populations in this third group.

2.2. Phytochemical profiles: flavonols and crocetin esters

In this study, the combined use of UV-vis (from HPLC-DAD) and mass (from LC-ESI-MS) spectra allowed the tentative of identifying several specialised metabolites belonging to the chemical classes of flavonoids and apocarotenoids, which can be considered among the most effective antioxidants in the human diet. Table 4 lists the content of flavonoids in the 15 samples of the wild *Crocus* species under study. Overall, 20 components were detected, belonging to the biochemical class of flavonols, a sub-class of flavonoids. The majority of flavonols were found as glycosylated compounds with only three aglycones, namely kaempferol, quercetin and isorhamnetin (Figure 2). The total amounts of these components ranged from 2.7 to 164.7 mg/g (Table 4). Population 12 (*C. siculus* – Acerone) was characterized by a noticeable number of flavonols (13 compounds) and the highest total amount (164.7 mg/g), followed by *C. neapolitanus* – Magnaudo (15) and Piani di Vacquarro (16), with the total amounts of 145.9 and 129.6 mg/g, respectively, and with 13 components each. On the contrary, *C. thomasii* – Sant'Angelo (14) exhibited a very low flavonol content (2.7 mg/g), as well as a much lower number of components (4). *C. biflorus* – Alessi (1) and Gibbola (2) showed

similar quantitative and qualitative traits. The nine samples belonging to *C. longiflorus* taxon displayed variegated compositional features (amount comprised between 18.3 and 99.7 mg/g, number of components ranging from #4 to #11).

Within the flavonol subclass, kaempferol derivatives were the most frequently appearing with 13 compounds followed by 6 quercetin derivatives, whereas only one isorhamnetin was detected in a few populations as aglycon. Components from #10 to #12 (tentatively identified as kaempferol di-hexoside) were present in all samples with a wide concentration range for one of its isomers (#12, Table 4), which was the most represented compound in *C. siculus* and in the two populations of *C. neapolitanus.* For the sake of comparison, Table 4 reports also the content of flavonols in a population of *C. sativus*, which was as low as 10 mg/g and it was represented by only three kaempferol derivatives. Considering quantitative and qualitative flavonoid profiles, none of the wild *Crocus* populations showed any similarity to saffron, with the partial exception of *C. thomasii* (population 14, see Table 4).

Relating to crocins, 24 components were detected in the wild *Crocus* populations under study (Table 5). All of them belong to a peculiar class of secondary metabolites, namely apocarotenoids, whose presence characterizes the most famous species of the genus, that is *C. sativus* (Gresta et al., 2008; Bagur et al., 2018). As broadly reported in literature, apocarotenoids are characterized by a variegated number of glucoside moieties on both sides of a C_{20} terpenic chain, as well as the presence of *trans* and *cis* isomers of the aforesaid chain (Figure 2). The total amounts of these components in our populations ranged from 19.8 to 604.0 mg/g, while the number of components was more uniform across populations with respect to flavonols, ranging from 14 to 17 (Table 5). The two *C. biflorus* populations showed similar quantitative and qualitative features as already

observed for the flavonoid profile. Populations belonging to *C. longiflorus* taxon, although characterized by similar qualitative traits (15-16 components) exhibited a wide range of variability in terms of total content (from 60.7 and 604.0 mg/g; Table 5). *C. siculus* was one of the populations with the lowest amount of crocins (36.1 mg/g) together with *C. thomasii* (24.2 mg/g) and the two *C. neapolitanus* populations, with 36.2 and 19.8 mg/g, respectively.

Noteworthy, four metabolites (#4, #6, #9 and #18, Table 5) were found in all samples even if with a wide range of concentrations. In particular, the compounds #6 and #9 are known as crocin-1 and crocin 2 and they represented the main apocarotenoids in saffron (Table 5) as shown by Gresta et al. (2008); these compounds were also found in many of the wild *Crocus* populations, particularly in those belonging to the *C. longiflorus* taxon (populations 3, 9 and 11, Table 5). Unlike flavonols, the amount of total crocins in saffron was significantly higher compared to all wild *Crocus* populations and, from a qualitative point of view, the crocin profile in saffron was very similar to that of *C. longiflorus* - Vaito (population 11).

The difference between populations in chemical profile appeared to be rather high, both in qualitative and in quantitative terms.

Figure S1 (supplementary material) shows the HPLC profiles, visualized at different wavelengths, for the aqueous extracts from some selected *Crocus* populations. The compositional data were submitted to PCA to obtain the biplot in Figure 3 that represents 95% of data variability and describes the similarities of populations in terms of chemical profiles. We see that the presence of the crocins #6, #9 and #18 is the typical characteristic of saffron, *C. longiflorus* Vaito, and, to a lower extent, *C. longiflorus* - Consolino and Alberi. On the other hand, *C. biflorus* (both populations) and *C. thomasii*

223 are mainly characterized by the crocin #2 and by the flavonoid #18. *C. siculus* and *C.*
224 *neapolitanus* (both populations) are mainly characterized by the flavonol #12. This is
225 further confirmed by the principal component analysis carried out using flavonols and
226 crocins separately (Figure S2; supplementary material).
227

228 ## 3. Discussion

229 *Crocus* is a well-known genus from ecological, horticultural, culinary and
230 pharmacological points of view (Sheidai et al., 2018). Saffron is the dried stigmas of *C.*
231 *sativus* being one of the most expensive spices in the world (Gresta et al., 2008; Sheidai
232 et al., 2018). Nonetheless, *Crocus* taxonomy is quite controversial and, especially in the
233 past, it was mainly based on morphological traits, as well as on chromosome number
234 (Harpke et al., 2013; Harpke et al., 2015; Sheidai et al., 2018; Anabat, 2022). Exploring
235 both morphological and chemical diversity within species and populations maybe an
236 important key for a better characterization and valorization of wild taxa. Although several
237 studies have focused on the classification of different wild *Crocus* taxa (Grilli Caiola et
238 al., 2004; Özdemir et al., 2008; Baghalian et al., 2010; Colasante, 2017), to date, the
239 availability of data about their phytochemical composition is very scarce.

240 Comparative analysis of morphological traits among populations and species
241 (Table 2 and Figure 1) allowed us to define the parameters that most discriminate the
242 different populations. The five *Crocus* species were clearly discriminated, except for *C.*
243 *thomasii* (14), which was found closer to *C. longiflorus* (especially the populations 6, 8
244 and 10). *C. longiflorus* - Radena (7) and, to a lower extent, *C. longiflorus* - Cane (5) were
245 different from the other populations of the same species, due to high values on Lsb and
246 Lpt. The morphological traits of *C. longiflorus* population 7 were in agreement with

247 previous findings by Colasante (2017) and Harpke et al. (2015), and fall within the
248 morphological variation of the species. The variability of morphological traits between
249 species was lower than that between populations, which means that plant morphology
250 may be strongly influenced by the environmental conditions (local soil and climatic
251 conditions) as well as by the genotypic factors, resulting from the genetic isolation of
252 fragmented populations and from geographical distances across the distribution range of
253 wild *Crocus* populations. Indeed, several authors have shown that phenotypic plasticity
254 is an important factor in determining the adaptive responses of plant species to changing
255 environments (Walter et al., 2020; 2022). Such an adaptability can enhance the spreading
256 of species to a large scale.

257 *Crocus* species are particularly rich in bioactive specialised metabolites
258 (Mikhailenko et al. 2019). Further investigations and particularly the use of more
259 exhaustive techniques such as mono-dimensional and bidimensional NMR are
260 compulsory in obtaining a complete identification of the metabolites hereby reported.
261 Flavonoid derivatives from *Crocus* species have been reported by several research groups
262 (Carmona et al., 2007; Gresta et al., 2008; Acra et al., 2010; Karimi et al., 2010; Šola et
263 al., 2018).

264 The content in phytochemicals in *Crocus* species is very important. In this regard,
265 crocins belong to C_{20} apocarotenoids and they are an important and peculiar class of
266 natural pigments typical of the *Crocus* genus (Gresta et al., 2008). These compounds, like
267 all carotenoids, are involved in a wide range of processes in plants, including growth and
268 development, responses to environmental stimuli, photosynthesis (as accessory
269 pigments), attracting pollinators and acting as signalling molecules for plant development
270 and mediating responses to environmental cues (Grilli Caiola and Canini, 2010).

A recent review investigated the content of phytochemicals in several *Crocus* species (Mykhailenko et al., 2019). The presence of about 170 specialised metabolites was detected, including carotenoids, flavonoids, anthocyanins, terpenoids, phenols carboxylic acids, etc., which were found at relatively high concentrations in style branches and in other plant parts, such as perianth, stamens, leaves, corms, mainly in *C. sativus* (Jadouali et al., 2017; Mykhailenko et al., 2019; Mottaghipisheh et al., 2020; Stelluti et al., 2021), but, to a lesser extent, also in other *Crocus* species. In this work, we detected 44 compounds, among which 20 belong to the group of flavonols and 24 to the group of crocins. A complete list of all the specialised metabolites with their diagnostic UV-vis and mass signals is given in Table S1 (Supplementary material). The aforesaid data allowed establishing the tentative identification of components by a combination of retention times, UV-vis absorbance profiles, mass spectra data, and when possible comparison with literature data.

In this latter respect, PCA showed several interesting putative groups, containing relatively similar populations (Figure 3). A first group was composed by *C. biflorus* and *C. thomasii* and it was characterized by low levels of the crocins #6, #9 and #18 and relatively high contents in crocin #2 and flavonols #15 and #18. A second putative group comprises the species *C. neapolitanus* and *C. siculus,* with low contents in crocins and relatively high contents in the flavonols #12 and #17. A third group contains *C. longiflorus*, where the populations are located on a gradient relating to the contents in the crocins #6, #9 and #18. In this group, the chemical profile of *C. longiflorus* 7 is relatively similar to that of the other populations of the same species (especially the populations 3, 5, 6 and 9), even though this population 7 had shown apparent differences in morphology. Between the two classes of compounds, we see that, with respect to crocins, flavonols

295 permitted a better discrimination of species, which appeared to be more closely related to
296 that obtained with the morphological traits (Figure S2 in supplementary materials).

297 It may be interesting to note that the classification tree arising from cluster
298 analyses based on both morphology and chemical profiles tend to put together in the same
299 group the populations belonging to the same species, with the only exception of the
300 populations 8 and 10, which are not classified together with the other populations of *C.*
301 *longiflorus*. In this respect, the classification tree based on only the morphological traits
302 seems to be less respectful of phylogenetic relationships (see Figure S3 in supplementary
303 material).

304 It is also interesting to compare the chemical profile of saffron (*C. sativus)* with
305 that of the wild species. In relation to flavonoids, literature references show some
306 similarities in relation to the glycosidic derivatives of kaempferol (Carmona et al., 2007;
307 Gresta et al., 2008). In this work, flavonol content in saffron was very low and the
308 chemical profile showed some similarities only with *C. thomasii*. On the other hand, the
309 apocarotenoids profile of the wild *Crocus* populations in this work appeared to be
310 characterized by several common components with *C. sativus*, although some wild
311 populations, particularly the two belonging to *C. biflorus* showed the presence of highly
312 glucosylated crocins with seven and six glucose moieties. To a lesser extent, this feature
313 was also found in two populations of *C. longiflorus* (4 and 9) and in *C. thomasii* (14), as
314 reported in Table 5. The presence of highly glucosylated crocins, with up to eight glucose
315 units, have been reported also for another wild *Crocus* species, namely *C. ancyrensis*,
316 also known as Ankara *Crocus*, that is endemic to Turkey (Ahrazem et al., 2015).

317 The variation of chemical profiles depending on populations may be ascribed to
318 the effect of environment conditions, which has been already described in previous works

(Carmona et al., 2007; Siracusa et al., 2010). Such a finding, in this work, appeared to be much more evident for crocins compared to flavonoids.

4. Conclusions.

Flower morphological traits were useful to discriminate *Crocus* species. However, these traits showed a rather high variability among populations of the same species and appeared to be under high environmental control.

The study of the phytochemical profile of the orange-red stigmas of wild taxa of *Crocus* led to the characterization of apocarotenoids (crocins) and flavonoids (flavonols). Our results confirmed that these taxa, in relation to saffron (*C. sativus*), may represent a good alternative rich source of these bioactive compounds. More in particular, the qualitative and quantitative content of flavonoids represented a distinctive and peculiar phytochemical characteristic of wild *Crocus* populations, as compared to saffron sample. Considering that the flavonoids are among the most important polyphenols, owing to a large number of medicinal benefits, their occurrence makes the wild *Crocus* species a very promising ingredient in the food and pharmaceutical industry.

The qualitative and quantitative composition in flavonols and crocins appeared to be rather peculiar for the different wild species of saffron and may contribute to a better determination of species. Therefore, our results showed that the combined use of morphological and phytochemical traits for taxonomic purposes lead to an improved characterisation of wild *Crocus* genotypes. Future studies should be planned to more precisely address this issue.

5. Experimental

343 *5.1. Plant materials*

344 In order to obtain a representative sampling of their diversity, a total of 16
345 populations belonging to *Crocus biflorus* Mill., *Crocus longiflorus* Raf., *Crocus*
346 *neapolitanus* (Ker Gawl.) Loisel., *Crocus siculus* Tineo ex Guss. and *Crocus thomasii*
347 Ten. (Figure 4) of the Iridiaceae family were studied. Flowers were collected in the 2017-
348 2018 years, during the flowering season, from different localities in Basilicata, Calabria
349 and Sicily (Italy) at an altitude ranging from 369 to 1780 m a.s.l. Over 300-flowers of
350 each species and population at the stage of anthesis were collected for phytochemical
351 characterization, and fifteen flowers per population were used for morphological traits.
352 The plant collection site (GPS coordinates and altitudes above mean sea level) and
353 collection date of species and populations are listed in Table 1. One accession of *C.*
354 *sativus* was intentionally inserted as an out group to validate the analysis of specialised
355 metabolites. Voucher specimens of all analysed populations and taxa were deposited for
356 future reference at the herbaria of the University of Catania (CAT).

357

358 *5.2. Morphological investigations*

359 In order to assess the characters of the 16 populations of wild *Crocus*, five floral
360 traits including length of perigone tube (Lpt) and of outer and inner perigone segments
361 (Lops and Lips, tepals), length of style branches (Lsb) and anther (Lanth) were measured
362 for 15 individual plants per site. Phenotypic traits were compared to assess the variability
363 between species and within population level and among spcies.

364

365 *5.3. Specialised metabolic analysis-general*

366 All solvents used were high-purity American Chemical Society (ACS) solvents
367 from VWR (Milan, Italy); acetonitrile and water (VWR, Milan, Italy) were of HPLC
368 grade. Pure reference standard kaempferol 3-*O*-glucoside and rutin (quecetin 3-*O*-
369 rutinoside) were obtained from Fluka (Milan, Italy). Pure *trans*-crocetin di-(β-D-
370 gentiobiosyl) ester (R^2 = 0.9985), in-house isolated from *C. biflorus* stigmas, was used as
371 external standard for pigment quantification (see paragraph 5.6).
372
373 *5.4. Sample preparation, qualitative and quantitative HPLC analyses*
374 *Crocus* style branches were dried at 30 °C for 72 h using a drying convection oven
375 (Termaks B8023, Bergen, Norway). Aliquots (10 mg) of each *Crocus* style branches
376 samples were finely chopped with a pestle and mortar, suspended in H_2O (2 mL) in a 4
377 mL amber vials (Chemtek Analytica, Milano, Italy) and kept for one hour, at room
378 temperature, in the dark under vigorous and continuous stirring in the shaker (200 *rpm*).
379 For each sample, it was considered an extractive duplicate. The resulting colored
380 suspension was then spin-dried at 3000 *rpm* (ALC PK 130 centrifuge, Milan Italy)
381 allowing to recover the supernatant yellow/orange solution. A small aliquot (250 uL) of
382 this solution was then brought to 1 mL with distilled water, filtered with PTFE filters (15
383 mm diameter, 0.45 μ pore size (Chemtek Analytica, Milan, Italy), put in a 2 mL amber
384 vial, and freshly analyzed for the determination of the specialised metabolites flavanols
385 and crocetin esters. The amount of sample of *C. siculus* – Piano Battagia (population 13,
386 Table 1) unfortunately was not sufficient to obtain reliable data.
387 Qualitative and quantitative analyses were carried out on an Ultimate 3000
388 'UHPLC focused' instrument equipped with a binary high-pressure pump, a Photodiode
389 Array detector, a Thermostated Column Compartment and an Automated Sample Injector

(Thermo Scientific, Chromatographic runs were performed using reverse-phase column (Gemini C_{18}, 250 × 4.6 mm, 5 μm particle size, Phenomenex, Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. The extracts from style branches *Crocus* were eluted with the following gradient of B (formic acid, 2.5% solution in acetonitrile) in A (2.5% solution of formic acid in water): 0 min: 10% B; 10 min: 100% B; 30 min: 10% B. The solvent flow rate was 1 mL/min, the temperature was kept at 25 °C, and the injector volume selected was 20 μL. DAD analyses were carried out in the range between 700 and 200 nm, registering the chromatograms at 260, 350 and 440 nm. Quantifications were carried out at 350 nm for flavonoids using calibration curves established with kaempferol 3-*O*-glucoside (standard concentration range from 0.0003 mg/mL to 0.0018 mg/mL; calibration curve equation y =11242347.85x – 0.1713; correlation coefficient R^2 = 0.9995) and rutin (standard concentration range from 0.00012 mg/mL to 0.00072 mg/mL; calibration curve equation y = 13216682.2x + 0,0109; correlation coefficient R^2 = 0.9998) for kaempferol and quercetin derivatives, respectively; rutin was used also to quantify isorhamnetin. Crocetin esters were quantified at 440 nm, in this case, the in-house isolated *trans*-crocetin di-(β-D-gentiobiosyl) ester (standard concentration range from 0.00065 mg/mL to 0.0039 mg/mL; calibration curve equation y = 14433542.75x + 3.0822; correlation coefficient R^2 = 0.9985) was used as external standard (see the corresponding paragraph).

5.5. HPLC/ESI/MS Analyses

In order to unambiguously identify the chromatographic signals and/or to confirm peak assignments, a series of HPLC/ESI-MS analyses were performed on a selected number of samples. The HPLC apparatus, solvent system, elution programs used were

414 the same as those described above, whilst ESI mass spectra were acquired by Thermo
415 Scientific Exactive Plus Orbitra MS (Thermo Fisher Scientific, Inc., Milan, Italy), using
416 a heated electrospray ionization (HESI II) interface. Mass spectra were recorded
417 operating in positive and negative ion mode in the *m/z* range of 120 – 1500 at a resolving
418 power of 25000 (full-width-at-half-maximum, at *m/z* 200, RFWHM), resulting in a scan
419 rate of >1.5 scans/sec when using automatic gain control target of 1.0×10^6 and a C-trap
420 inject time of 250 ms under the following conditions: capillary temperature 300 °C,
421 nebulizer gas (nitrogen) with a flow rate of 60 arbitrary units; auxiliary gas flow rate of
422 10 arbitrary units; source voltage 3 kV; capillary voltage 82.5 V; tube lens voltage 85 V.
423 The Orbitrap MS system was tuned and calibrated in positive ion modes, by infusion of
424 solutions of a standard mixture of sodium dodecyl sulfate (Mr 265.17 Da), sodium
425 taurocholate (Mr 514.42 Da) and Ultramark (Mr 1621 Da). Data acquisition and analyses
426 were performed using the Excalibur software.
427
428 *5.6. Isolation of trans-crocetin di-(β-D-gentiobiosyl) ester from C. biflorus*
429 A suitable amount (425 mg) of *C. biflorus* style branches was finely powdered
430 and suspended in H_2O (100 mL) in a 250 mL laboratory flask. The heterogeneous mixture
431 was then kept for one hour at room temperature, in the dark and under vigorous (200 rpm)
432 and continuous stirring in a shaker.
433 The resulting suspension was filtered under vacuum allowing us to recover a
434 yellow/orange solution containing crocus pigments; the colorless solid residue was
435 discarded. The solution was lyophilized using a Telstar Lyoquest freeze drier: the sample
436 was mixed with 200 mL of water and, after 45 min of freezing, the temperature was

437 brought to - 80 °C and maintained for 36 h. With this procedure, 150 mg of stigma extract,
438 rich in crocetin esters, was obtained.

439 For the isolation of the *trans*-crocetin di-(β-D-gentiobiosyl) ester, the lyophilized
440 extract was purified through open column chromatography using RP-18 as stationary
441 phase and a linear gradient of B (formic acid 2.5% in acetonitrile) in A (2.5% solution of
442 formic acid in water) as eluent. The progress of the column was monitored by HPLC.
443 After the removal of the solvents *in vacuo*, *trans*-crocetin di-(β-D-gentiobiosyl) ester was
444 obtained (37 mg) as pure compound (purity over 95%) as estimated by HPLC and further
445 confirmed by ^{1}H-NMR data through comparison with existing literature (Ahrazem et al.,
446 2015).

447

448 *5.7. Data analysis*

449 In order to evaluate the variability across species, across populations within
450 species and across individual plants within populations and species, a linear mixed effect
451 model was fitted to the observed data for each morphological variable and the variance
452 components for the above-mentioned effects were estimated by using REstricted
453 Maximum Likelihood estimation (REML). For morphological traits and chemical
454 profiles, Principal Component Analyses (PCA) on the correlation matrix were used to
455 characterize the similarity among population (all species). Results were reported on a
456 'distance' biplot (Oksanen et al., 2020). Cluster analysis was used to group the accessions
457 (all species) according to the similarity of morphological traits; analysis was based on
458 euclidean distances together with unweighted pair mean group averages as the clustering
459 algorithm. All analyses were performed by using the R statistical environment (R core

team, 2021), together with the packages 'lme' (Pinheiro and Bates, 2000), 'MASS' (Venables and Ripley, 2002) and 'vegan' (Oksanen et al., 2020).

Author Contributions: conceptualization, A.C., G.R; methodology, A.C., G.R., L.S. and A.O.; investigation, A.C., C.I., R.G., G.S., L.P. and L.S; data curation, A.C., L.S. and A.O.; formal analysis, A.O. and L.P.; writing-original draft, A.C., A.O., F.G. and G.R.; writing-review and editing, all authors; funding acquisition, A.C. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data.

Figure S1. HPLC profiles of selected *C. biflorus* (**A**), *C. longiflorus* (**B**) and *C. neapolitanus* (**C**) extracts. Flavonoids (grey traces) are visualized at 350 nm, whilst crocins (black traces) are visualized at 440 nm. See Table 4, Table 5 and Table S1 for peak numbering and text for further details.

Figure S2. Biplot from Principal Component Analysis based on the content of flavonols (up) and crocins (down). See Table 1 for the coding of populations, Table 4 and Table 5 for the coding of compounds.

Figure S3. Dendrograms from cluster analysis for *Crocus* populations (See Table 1 for the coding of populations). **A**: only morphological traits; **B**: morphological and phytochemical traits

Table S1. Peak list and diagnostics for the Crocus samples extracts object of this stud. For peak number see also Table 4 and Table 5, and Figure S1 for chromatograms.

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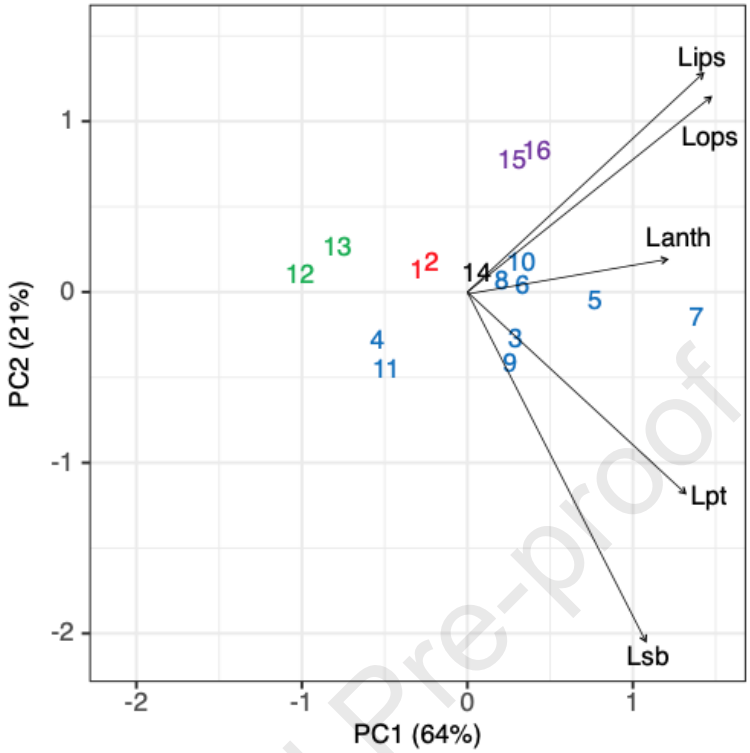
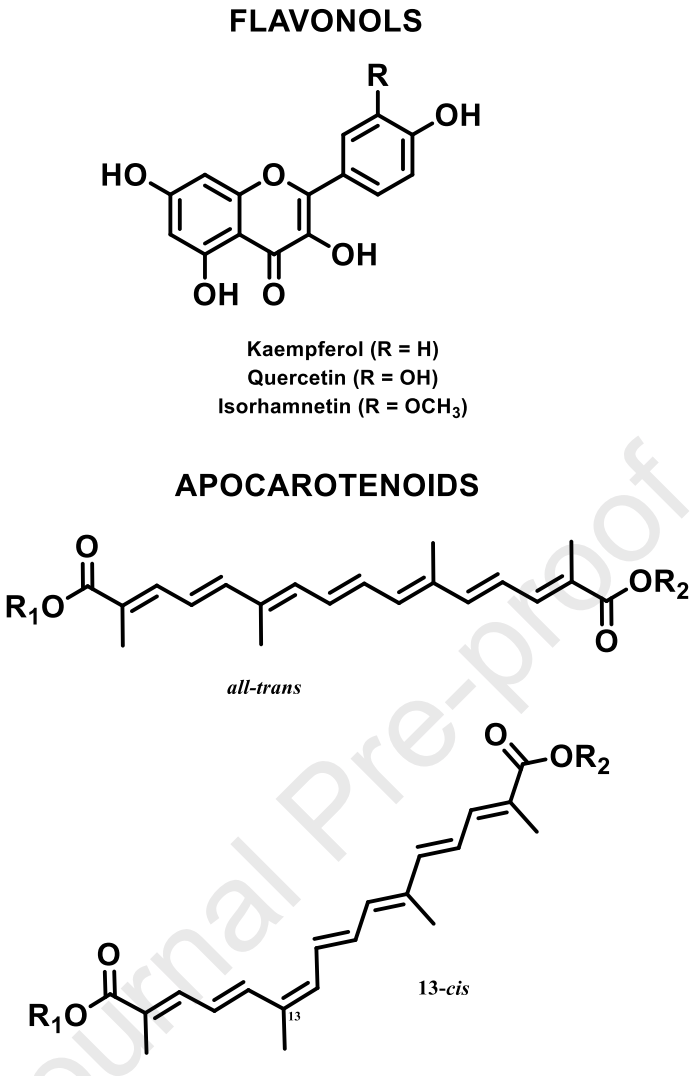


Figure 1. Biplot from Principal Component Analysis for the floral traits in the sixteen *Crocus* populations. Lops and Lips: outer and inner perigone segments; Lsb: length of style-branches; Lpt: Length of perigone tube; Lanth: length of anther of the 15 Crocus samples. See Table 1 for the coding of populations.



R1 = R2 = H *trans* and *cis* crocetin

R_1 and R_2= n-glucose moieties, crocins

Figure 2. General molecular structures of flavonoids (flavonols) and apocarotenoids (crocins) characterised in the stigmas of wild *Crocus* samples and saffron.

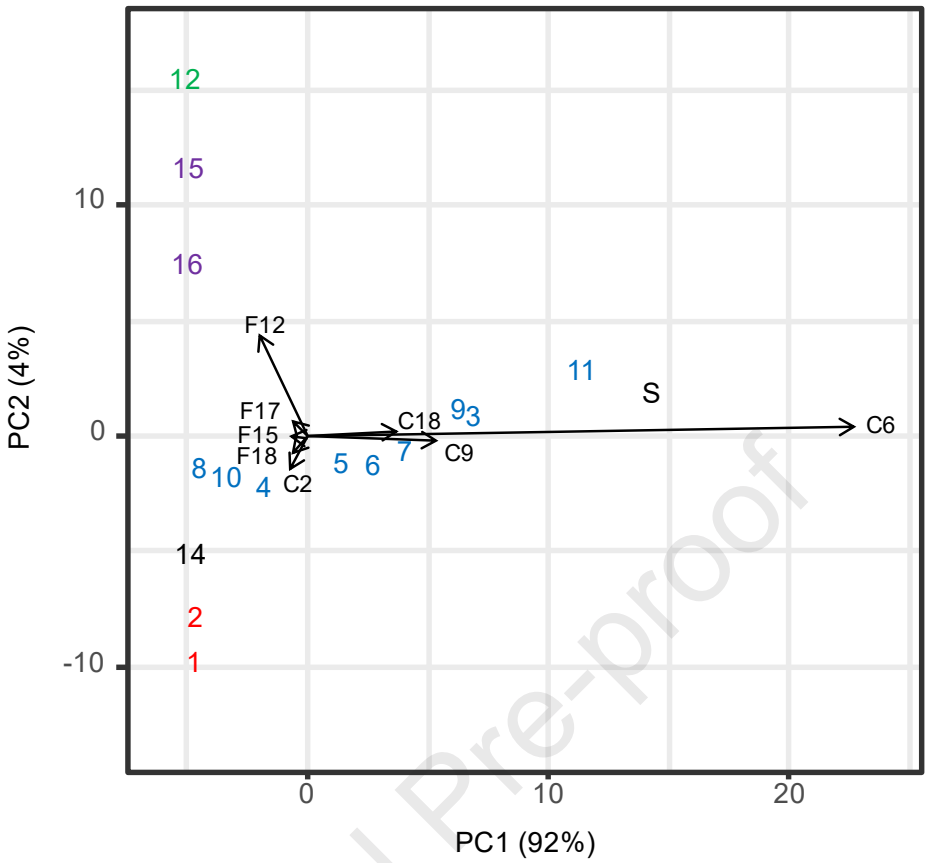


Figure 3. Biplot from Principal Component Analysis for the phytochemical components (flavonols and crocins) for fifteen wild *Crocus* samples and saffron. All components were used for the analysis, but only the ones with the longest vectors were represented in the graph. See Table 1 for the coding of populations (S = saffron) and Tables 4 and 5 for the coding of phytochemicals.

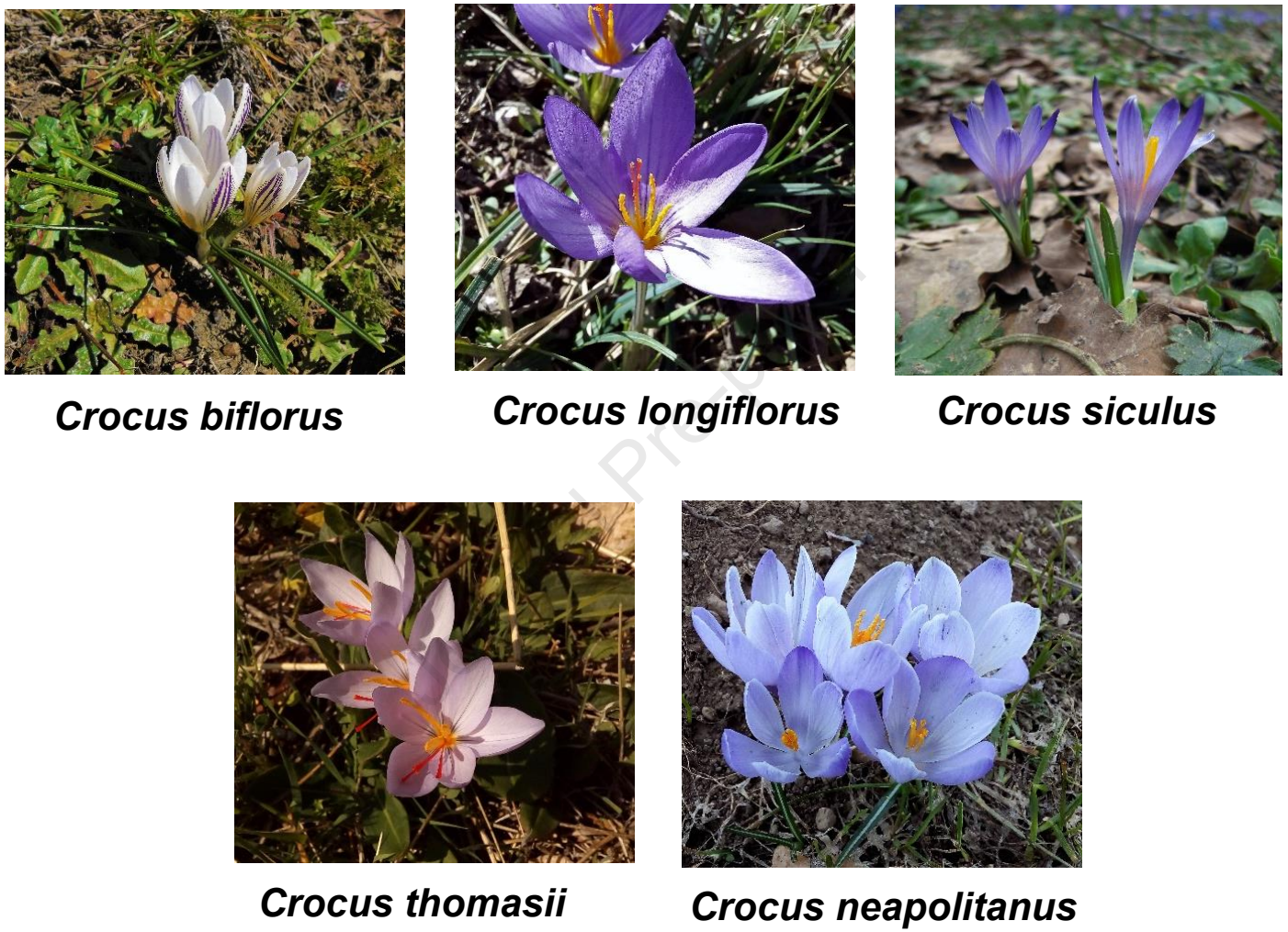


Figure 4. Wild *Crocus* taxa collected in Southern Italy: *Crocus biflorus*; *C. longiflorus; C. siculus*; *C. thomasii*; *C. neapolitanus*.

Table 1. Native populations of *Crocus* species collected in Southern Italy (Basilicata, Calabria, Sicily)

Population Number	*Species*	Collection Date	Geographical origin, District, Province, Locality	Habitat	Coordinates WGS84	Altitude m a.s.l.	Voucher No.
1	*Crocus biflorus*	30/01/18	Nebrodi mountains Capizzi - Messina Serra d'Alessi	pasture land on clays with arenites	37° 53' 18" N 14° 31' 14" E	1380	20180112
2	*Crocus biflorus*	30/01/18	Nebrodi mountains Cesarò - Messina Gibbola	pasture land on clays with arenites	37° 50' 20" N 14° 34' 39" E	1100	20180111
3	*Crocus longiflorus*	23/10/17	Iblei mountains Buccheri - Siracusa Alberi	oak wood on vulcanites	37° 08' 15" N 14° 52' 24" E	600	20171004
4	*Crocus longiflorus*	24/11/17	Sicani mountains Godrano - Palermo Bosco della Ficuzza, Busambra	deciduous oak forest	37° 51' 39" N 13° 25' 13" E	1003	20171109
5	*Crocus longiflorus*	15/11/17	Etna volcano Pedara - Catania Monte Salto del Cane	chestnut wood	37° 40' 26" N 15° 02' 15" E	1290	20171108
6	*Crocus longiflorus*	15/11/17	Etna volcano Ragalna - Catania Monte Elici	deciduous oak forest	37° 39' 55" N 14° 57' 37" E	1157	20171107
7	*Crocus longiflorus*	31/10/17	Aspromonte mountains Reggio Calabria Radena	pasture land	38° 06' 00.31" N 15° 45' 25.5" E	872	20171005
8	*Crocus longiflorus*	18/10/17	Nebrodi mountains Cesarò - Messina Vallone Ruvolo	mountain pasture land	37° 53' 11" N 14° 41' 30" E	1325	20171002
9	*Crocus longiflorus*	04/11/17	Serre Stilo - Reggio Calabria Monte Consolino	xerophilous grasslands	38° 28' 45" N 16° 27' 44" E	626	20171106
10	*Crocus longiflorus*	18/10/17	Nebrodi mountains Cesarò - Messina Torrente Torto	mountain pasture land	37° 53' 50" N 14° 39' 50" E	1397	20171001
11	*Crocus longiflorus*	23/10/17	Iblei mountains Licodia Eubea - Catania Bosco Vaito	cork forest on red sand	37° 08' 52" N 14° 36' 34" E	369	20171003
12	*Crocus siculus*	10/05/18	Nebrodi mountains Cesarò - Messina Monte Soro - Acerone	beech forest clearings	37° 55' 54.21" N 14° 41' 28.09" E	1780	20180516
13	*Crocus siculus*	09/04/18	Madonie mountains Petralia Sottana - Palermo Piano Battaglia	beech forest clearings	37° 52' 27.13" N 14° 01' 09.36" E	1554	20180415
14	*Crocus thomasii*	03/11/17	Pollino Mountains Castrovillari - Cosenza S. Lucia - Monte S. Angelo	stone pine forest clearings	39° 49' 12" N 16° 11' 41" E	425	20171110
15	*Crocus neapolitanus*	24/04/18	Sila Grande mountains San Giovanni in Fiore - Cosenza Serra Magnaudo	beech and laricio pine forests clearings	39° 16' 29.01" N 16° 29' 36.28" E	1639	20180414
16	*Crocus neapolitanus*	23/04/18	Pollino Mountains Fardella - Potenza Piani di Vacquarro	beech clearings	39° 55' 45.59" N 16° 10' 15.61" E	1425	20180413

Table 2. Morphological traits of the five wild *Crocus* species and populations under investigation.

#	Species	Length (mm)*				
		perigone tube (Lpt)	outer perigone segments (Lops)	inner perigone segments (Lips)	anthers (Lanth)	style-branches (Lsb)
1	*Crocus biflorus (*Alessi)	58.4 (3.3)	30.7 (1.0)	28.0 (1.0)	10.7 (0.2)	8.3 (0.4)
2	*Crocus biflorus* (Gibbola)	51.2 (3.1)	31.2 (1.2)	28.7 (1.2)	11.1 (0.2)	9.9 (0.7)
	***Crocus biflorus* (Mean)**	**54.8 (2.3)**	**31.0 (0.8)**	**28.4 (0.7)**	**10.9 (0.2)**	**9.1 (0.4)**
3	*Crocus longiflorus* (Alberi)	87.6 (4.5)	32.4 (1.1)	29.3 (1.2)	12.5 (0.4)	14.9 (0.9)
4	*Crocus longiflorus* (Ficuzza)	70.9 (2.3)	25.7 (1.0)	23.6 (1.0)	10.1 (0.4)	9.6 (0.4)
5	*Crocus longiflorus* (Cane)	92.1 (5.8)	37.8 (1.5)	34.0 (1.3)	13.8 (0.5)	15.8 (0.8)
6	*Crocus longiflorus* (Elici)	69.4 (3.3)	34.7 (0.9)	31.6 (1.0)	13.1 (0.4)	13.6 (0.8)
7	*Crocus longiflorus* (Radena)	135.9 (4.1)	42.7 (1)	39.0 (1.1)	12.8 (0.3)	17.9 (1.1)
8	*Crocus longiflorus* (Ruvolo)	79.0 (4.6)	35 (1.6)	31.0 (1.4)	11.4 (0.4)	11.3 (0.5)
9	*Crocus longiflorus* (Consolino)	68.1 (4.1)	30.3 (0.9)	26.4 (0.9)	15.4 (0.5)	17.8 (0.8)
10	*Crocus longiflorus* (Torto)	82.1 (4.5)	35.6 (1.4)	32.3 (1.3)	11.9 (0.4)	9.9 (0.9)
11	*Crocus longiflorus* (Vaito)	65.9 (2.8)	24.5 (0.7)	22.0 (0.8)	11.2 (0.4)	12.1 (0.8)
	***Crocus longiflorus* (Mean)**	**83.5 (2.2)**	**33.2 (0.6)**	**29.9 (0.6)**	**12.5 (0.2)**	**13.6 (0.4)**
12	*Crocus siculus* (Acerone)	40.9 (1.3)	25.4 (0.8)	23.0 (0.7)	7.9 (0.3)	4.5 (0.9)
13	*Crocus siculus* (Battaglia)	48.0 (1.9)	27.9 (0.6)	25.2 (0.5)	8.7 (0.3)	3.4 (0.2)
	***Crocus siculus* (Mean)**	**44.4 (1.3)**	**26.6 (0.5)**	**24.1 (0.5)**	**8.3 (0.2)**	**3.9 (0.4)**
14	*Crocus thomasii* (Sant'Angelo)	71.4 (2.2)	32.7 (1.1)	29.0 (1.0)	14.8 (0.4)	10.0 (0.4)
15	*Crocus neapolitanus* (Magnaudo)	51.3 (2.0)	37.8 (1.0)	35.0 (0.9)	13.6 (0.5)	4.9 (0.3)
16	*Crocus neapolitanus* (Vacquarro)	54.1 (1.8)	37.5 (1.3)	34.7 (1.0)	14.4 (0.4)	4.5 (0.3)
	***Crocus neapolitanus* (Mean)**	**52.7 (1.3)**	**37.6 (0.8)**	**34.8 (0.7)**	**14.0 (0.3)**	**4.7 (0.2)**
	Overall mean for species	*61.3*	*32.2*	*29.2*	*12.1*	*8.3*

*In brackets standard error

Table 3. Restricted maximum likelihood (REML) method of variance component estimates based on morphological traits (perigone, style branches and anther samples) for the species and accessions under investigation.

Source of variability	Length (mm)				
	perigone tube (Lpt)	outer perigone segments (Lops)	inner perigone segments (Lips)	anthers (Lanth)	style branches (Lsb)
Among species	207.6	1.8	2.4	5.3	14.9
Among populations (within species)	312.7	24.1	20.5	1.7	6.9
Among genotypes (within population)	177.8	18.1	16.4	2.2	6.8

Table 4. Flavonoid content of the *Crocus* populations collected in the two-years period 2017-2018.

	Flavonoids	*1 - Crocus biflorus Alessi 2018*	*2 - Crocus biflorus Gibbola 2018*	*3 - Crocus longiflorus Alberi 2017*	*4 - Crocus longiflorus Ficuzza 2017*	*5 - Crocus longiflorus Cane 2017*	*6 - Crocus longiflorus Elici 2017*	*7 - Crocus longiflorus Radena 2017*	*8 - Crocus longiflorus Ruvolo 2017*	*9 - Crocus longiflorus Consolino 2017*	*10 - Crocus longiflorus Tortoo 2017*	*11 - Crocus longiflorus Vaito 2017*	*12 - Crocus siculus Acerone 2017*	*14 - Crocus thomasii Sant'Angelo 2017*	*15 - Crocus neapolitanus Maganudo 2018*	*16 - Crocus neapolitanus Vacquarro 2018*	*Crocus.sativus (saaffron) Buccheri 2018 (reference)*
#	**Compound**	**mg/g**															
1	Kaempferol di-hexoside derivative 1												0.3	0.3	0.9	0.2	0.9
2	Kaempferol di-hexoside derivative 2													0.6	0.4		0.5
3	Quercetin di-hexoside	3.1	2.7						1.8		1.2		0.2			1.2	
4	Quercetin di-hexoside 1						1.1	0.9	1.0		1.8	1.5	1.3		1.2		
5	Kaempferol di-hexoside derivative 2												0.4		0.8	0.5	
6	Kaempferol di-hexoside derivative 3	9.7	6.7									0.9			3.7	1.7	
7	Quercetin derivative 1				1.0				2.3		2.1		1.1				
8	Quercetin derivative 2									3.0							
9	Quercetin di-hexoside 3				2.4	2.9	3.4			5.7			2.5		2.1	3.1	
10	Kaempferol di-hexoside isomer 1	0.6	0.5	6.4	10.5	9.6	5.9	6.2	18.9	13.4	16.2	12.8	17.1	1.0	21.3	12.4	
11	Kaempferol di-hexoside isomer 2			3.8	2.1	2.5	1.5	1.6	3.6	2.7	2.6	3.3					8.2
12	Kaempferol di-hexoside isomer 3			5.5	5.1	5.4	3.2	5.0	8.4	7.2	8.6	7.1	105.7		80.8	56.4	
13	Kaempferol derivative MW = 610	1.3	1.4	11.5	9.4			8.5	11.4		9.3	7.7	6.4		8.0	5.9	
14	Quercetin rutinoside							10.4			1.1					5.2	
15	Kaempferol 3-*O*-rutinoside	0.3	0.4		12.5				35.9		40.8		10.5		2.7	6.6	
16	Kaempferol 3-*O*-glucoside								1.6		0.6			0.8			
17	Kaempferol 3-*O*-rutinoside derivative 1	0.5	0.4						14.7				6.4		17.5	32.2	
18	Kaempferol 3-*O*-rutinoside derivative 2	34.2	35.2					1.0			5.4		11.2				
19	Kaempferol 3-*O*-glucoside derivative 1	0.7	0.7			2.6	3.3						1.7		2.0	1.7	
20	Isorhamnetin														4.5	2.7	
	total	**50.4**	**47.9**	**27.3**	**42.9**	**23.0**	**18.3**	**33.5**	**99.7**	**32.0**	**89.8**	**33.2**	**164.7**	**2.7**	**145.9**	**129.6**	**9.6**

Table 5. Apocarotenoids (crocins) content in the *Crocus* populations collected in the two-years period 2017-2018.

#	Apocarotenoids	*1 - Crocus biflorus Alessi*	*2 - Crocus biflorus Gibbola*	*3 - Crocus longiflorus Alberi*	*4 - Crocus longiflorus Ficuzza*	*5 - Crocus longiflorus Cane*	*6 - Crocus longiflorus Elici*	*7 - Crocus longiflorus Radena*	*8 - Crocus longiflorus Ruvoloo*	*9 - Crocus longiflorus Consolino*	*10 - Crocus longiflorus Torto*	*11 - Crocus longiflorus Vaito*	*12 - Crocus siculus Acerone*	*14 - Crocus thomasii Sant'Angelo*	*15 - Crocus neapolitanus Maganudo*	*16 - Crocus neapolitanus Vacquarro*	*Crocus sativus (saffron) Buccheri 2018 (reference)*
	Compound*	**mg/g dried stigmas**															
1	crocin (MW 1462)	4.3	2.4														
2	crocin (MW 1300)	57.6	33.3		4.1					6.1				13.1			
3	crocin (MW 1300)	1.7	1.4											0.2			
4	*trans*-crocetin (β-D-triglucosyl)-(β-D-gentiobiosyl) ester (**t-5tG**)	7.3	4.5	12.5	11.4	12.9	20.1	19.9	3.0	21.6	2.6	15.2	2.9	3.4	7.4	2.4	4.2
5	*trans*- crocetin (β-D-neapolitanosyl)-(β-D-gentiobiosyl) ester (**t-5nG**)	28.5	19.4											1.5			
6	*trans*-crocetin di-(β-d-gentiobiosyl) ester (**t-4GG**)	18.0	18.6	271.2	74.8	144.3	175.8	211.9	19.5	283.3	36.2	382.2	3.9	1.5	5.4	3.6	441.8
7	*trans*-4 glc isomer (**t-4g**)												4.6	0.4	3.6	1.2	
8	*cis*-5 glc isomer-1 (**c-5g**)	9.7											0.5	1.7	2.2	0.5	
9	*trans*-crocetin (β-D-gentiobiosyl)-(β-D-glucosyl) ester (**t-3Gg**)	3.2	2.5	84.7	33.0	63.3	69.7	66.7	9.8	51.1	12.2	65.3	1.1	0.9	3.8	2.6	120.7
10	*cis*-crocetin (β-D-neapolitanosyl)-(β-D-gentiobiosyl) ester (**c-5nG**)	5.4	3.6														2.4
11	*cis*-5 glc-isomer-2 (**c-5g**)	1.7	1.1		1.5	1.5	2.2	3.0	1.0	4.0	1.0	2.6	0.8	0.4	1.1		
12	*trans*-5 glc isomer (**t-5g**)			6.9	5.1	6.8	4.7	5.6	4.1	2.2	5.6	5.0	0.7	0.1	0.8	0.2	
13	*trans*-crocetin (β-D-neapolitanosyl)-(β-D-glucosyl) ester (**t-4ng**)	3.3	3.1	17.0	9.9	17.2	19.1	15.1	3.4	7.1	3.0	7.8			1.9	1.3	4.3
14	*cis*-crocetin di-(β-D-gentiobiosyl) ester (**c-4GG**)			35.6	14.6	26.0	23.7	30.9	5.4	50.5	6.8	48.2	0.3	0.2	1.2	0.9	31.6
15	*trans*-crocetin (β-D-gentiobiosyl) ester (**t-2G**)	2.7	2.2														
16	*cis*-3 glc isomer (**c-3g**)			0.9	0.3	0.5	0.4	0.6	0.5	0.7	0.4	0.9	1.0		0.2	0.2	0.8
17	*cis*-crocetin (β-D-gentiobiosyl)-(β-D-glucosyl) ester (**c-3Gg**)			11.8	6.6	10.1	9.1	9.8	2.3	8.9	1.9	7.8	0.9	0.10	1.0	0.9	13.8
18	*trans*-crocetin di-(β-D-glucosyl) ester (**t-2gg**)	3.0	3.2	80.6	30.4	42.6	48.8	45.5	8.7	34.3	12.3	60.0	12.9	0.37	4.8	4.2	74.6
19	*cis*-crocetin (β-D-gentiobiosyl) ester (**c-2G**)			2.3	1.6	2.1	1.8	1.7	0.5	1.0	0.4	0.7	0.8		0.4	0.5	1.0
20	*cis*-2 glc isomer (**c-2g**)												0.3		0.2	0.1	
21	*trans*-crocetin (β-D-glucosyl) ester (**t-1g**)	0.4	0.4	5.7	6.6	9.8	9.5	7.3	2.0	4.5	1.9	6.1	1.6		1.2	1.0	5.7
22	*cis*-crocetin di-(β-D-glucosyl) ester (**c-2gg**)			2.1	0.8	0.9	1.2	1.4	0.4	1.2	0.5	1.9		0.01			2.8
23	*cis*-crocetin (β-D-glucosyl) ester (**c-1g**)			0.5	0.2	0.4	0.3	0.2	0.1	0.2	0.1	0.2	0.2	0.03	0.1	0.1	0.1
24	*trans*-crocetin	T	0.1	0.9	0.6	0.8	0.8	0.34	0.1	0.4	0.1	0.4	1.4				0.3
	Total	**146.8**	**95.6**	**532.6**	**201.5**	**339.1**	**387.2**	**419.9**	**60.7**	**477.0**	**84.8**	**604.0**	**36.1**	**24.17**	**36.17**	**19.8**	**704.1**

*the abbreviations of crocins with known and tentative structures are reported in parenthesis (D'Archivio et al., 2016; Mykhailenko et al., 2019); T = trace (< 0.05 mg/g).

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HIGHLIGHTS

- Morphological and phytochemical traits effectively characterized wild Crocus genotypes
- Lengths of perigone tube, tepals, anthers and style branches discriminated Crocus taxa
- Twenty flavonols and twenty-four crocins have been quantified in the Crocus extracts
- Wild Crocus taxa may be an alternative rich source of crocins and flavonols

An integrated approach for the characterization of wild *Crocus* species adopting phenotypical and phytochemical traits

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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