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Research Paper

Exploring *Crocus Sativus* L. cultivation in new areas of North-Western Himalayas: The quality of saffron is location dependent

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ABSTRACT

Crocin and safranal are the major compounds responsible for saffron's exclusive colour and aroma, respectively, and these compounds are the determining parameters to assess saffron quality. In the present study, 18 saffron trails were set up across the North-Western Himalayan region inside Jammu and Kashmir, India. Most of the trials were laid in regions with no documented evidence of saffron cultivation and were therefore outside the traditional saffron growing area. The objective was to assess the effect of location on the saffron colour and aroma, and compare it with saffron obtained in the traditional area. The average values for crocin ranged between 28.40 to 48.96 mg/g and that of safranal ranged between 0.85 to 1.18 mg/g. Many sites located outside the traditional areas have shown promising results, and among these Malangpora (Pulwama), Haran (Budgam), Benhama (Ganderbal), Shalimar (Srinagar) and Mirgund (Baramulla) were at par with the traditional area (Pampore) in terms of crocin content. Similarly, for safranal content (aroma) Pampore (Control) was the best and was closely followed by Mirgund (Baramulla), Malangpora (Pulwama), Benhama (Ganderbal) and Shalimar (Srinagar) which were statistically at par with each other. Hence it can be concluded that there is a scope of growing good quality saffron outside traditional areas.

Key words: Crocin, *Crocus sativus*, HPLC, non-traditional areas, quality, saffron, safranal

Saffron (*Crocus sativus* L.) from the Iridaceae family is a sterile triploid herb with purple flowers. Red-dried stigmas are mostly used in cooking for their flavouring and colouring properties, as a dye and used in medicines (Husaini et al., 2021; Husaini & Wani, 2020; Rios et al., 1996). The commercial production of saffron is limited to Iran, Italy, Spain, Azerbaijan, Greece, India and Turkey (Husaini et al., 2013; Kafi et al., 2018). Saffron cultivation is adapted to temperate and sub-tropical regions of the world with variable soil compositions (Negbi, 1999). In India, commercial saffron production is limited to Jammu and Kashmir (J&K) and plays a vital part in the agriculture-based green-economy. Saffron covers about 4% of the total cultivated area in the Kashmir valley of India. Saffron is mainly cultivated in the plateaus of Pulwama (74.64%), Budgam (16.13%), Srinagar (6.68%), and Kishtwar (2.50%) districts (Husaini, Hassan, et al., 2010; Husaini, Kamili, et al., 2010). Saffron cultivation in Kashmir faces a serious crisis, which is evident from its dwindling share in global production. In 2020 India ranked 4th in saffron imports accounting for 9.71% of the world's imports, which is worth 23 million US $ *(https://trendeconomy.com/data/commodity_h2/091020).* Climate change is also emerging as a big challenge to saffron cultivation (Husaini, 2014). Untimely and early snowfall in the 1st week of November has been causing the loss of flowers in traditional areas of J&K, making the situation worse (Husaini & Wani, 2020). Saffron contains more than 150 volatile aroma-yielding compounds. Saffron quality depends on the concentration of its three major metabolites crocin, picrocrocin, and safranal, providing saffron's exclusive color, taste, and aroma, respectively (Farrokhi et al., 2021; Maggi et al., 2010; Rezaee & Hosseinzadeh, 2013). Several methods of determining saffron quality have been described (Carmona et al., 2006; Tarantilis et al., 1995). The quality analysis of saffron samples worldwide indicated variation in saffron quality, mostly

depending on drying, extraction, and analysis (Kanakis et al., 2004; Lozano et al., 2000; Zareena et al., 2001). Furthermore, there is a strong inverse correlation with adulteration (percentage) of the samples used for quality analysis (Husaini et al., 2022). The International Organization Standardization recommends UV–vis spectrophotometry (ISO-3632-1/2, 2003) method of estimating saffron quality. Despite the rapidness of this method, the reliability is sometimes disputed as the degree of specificity is lower than in other analytical methods. In the present study, the high-performance-liquid chromatography (HPLC) method was employed for determining saffron quality (crocin and safranal). This procedure is a rapid, accurate, cost-effective, versatile, quantitative, sensitive, and efficient method for the quality analysis of these compounds (Alonso et al., 2001; Dong, 2013).

A recent study discussed the potential of cultivating organic saffron in the kitchen gardens of five districts (Anantnag, Budgam, Ganderbal, Pulwama & Srinagar) in Kashmir, mostly outside the traditional saffron belt (Husaini & Wani, 2020). The current research is the first-ever study conducted on estimating saffron quality cultivated in ten Kashmir districts and six Jammu districts in the North-western Himalayan region. The purpose is to assess the potential of saffron cultivation in the neglected and marginal areas of the Trans-Himalayan region of Jehlum and Chenab valleys of Jammu and Kashmir, India.

MATERIALS AND METHODS

Location and layout: Eighteen (18) sites, each having an area of 253 m^2 (half kanal), across Jammu and Kashmir were selected for saffron cultivation (Table 1; Fig 1). In May-June 2018, the field was ploughed two to three times to the depth of about 30 cm using a tractor, at approximately 20-30 days intervals. In August (10 to 15th), levelling and hoeing operations were carried out, and fields were pulverized 3-4 times. Well-decomposed farmyard manure (14 tonnes/ha) was applied and thoroughly mixed into the soil before the last tillage operation. Weeds, dead plant material, and stones were removed from the field. The field was laid out into 2.5m × 7.5m beds with deep drainage channels on the sides. Planting was done at 10 × 15 cm spacing and depth of 15 cm with 1 corm/ditch. Corms weighing above 8g were planted @ 6 tonnes/ ha (approximately 600,000 corms/ha). Corm density/m^2 was 67. Vermicompost @ 1 tonne/ha was also added into the field. In addition, chemical fertilizers supplying Urea (184 kg/ha), MOP (195.6 kg/ha) and DAP (132 kg/ha) were also added to the fields.

Table 1: Geographical coordinates of the eighteen saffron sites

Location code	Location name	Altitude (m amsl)	Latitude	Longitude
S_0	Pampore (Control)	1593 m	32.88° N	74.92° E
S_1	Dooru, Anantnag (KVK)	1773 m	33.43° N	75.09° E
S_2	Malangpora, Pulwama (KVK)	1630 m	32.88° N	74.92° E
S_3	Balpora, Shopian (KVK)	1970 m	33.72° N	74.83° E
S_4	Kulangam, Kupwara (KVK)	1658 m	34.32° N	74.15° E
S_5	Haran, Budgam (KVK)	1520 m	34.0° N	75.02° E
S_6	Benhama, Ganderbal (KVK)	1784 m	34.21° N	74.77° E
S_7	Shalimar, Srinagar (FoH)	1625 m	34.8° N	74.51° E
S_8	Budhal, Rajouri	1926 m	33.37° N	74.63° E
S_9	Jalian Mandi, Poonch	1440 m	32.44° N	74.48° E
S_{10}	Mahore, Reasi	1582 m	33.19° N	74.50° E
S_{11}	Shiva, Doda	2215 m	33.35° N	74.32° E
S_{12}	Mir, Udhampur	1761 m	33.04° N	75.09° E
S_{13}	Gandhri, Ramban	1600 m	33.13° N	75.10° E
S_{14}	Mirgund, Baramulla (TSRI)	1584 m	33.97° N	74.76° E
S_{15}	Pombay, Kulgam (KVK)	1841 m	33.38° N	75.01° E
S_{16}	Shuhama, Ganderbal (KVK)	1595 m	35.30° N	75.15° E
S_{17}	Pochal, Kishtwar	1557 m	35.33° N	75.74° E

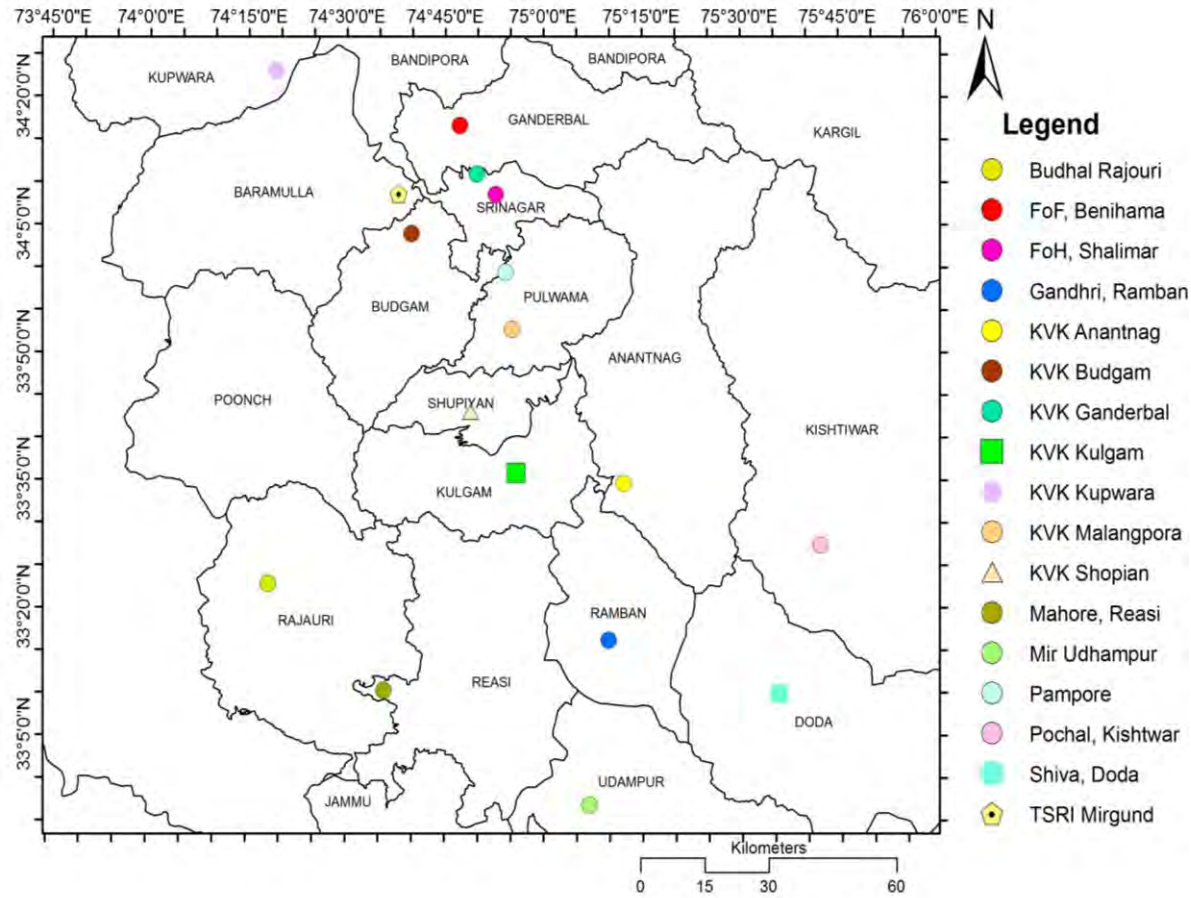

Fig. 1: Saffron cultivation trials at eighteen geo-tagged sites across Jammu and Kashmir, India

Flower harvesting: In the third year of flowering (2020), the saffron flowers of each experimental plot were picked by hand (wearing gloves). The flowers were collected two days post-anthesis as it was the most appropriate stage for metabolite accumulation (Husaini *et al.,* 2018); Haq *et al*. 2022). The stigmas were separated from flowers, shade dried for 3-4 days at room temperature of 25°C ± 2°C as is commonly practised by farmers. The stigmas were stored at 4°C and analyzed for crocin and safranal content after 2 weeks of storage.

Crocin and safranal estimation: Crocin and safranal standards were purchased from Sigma (Germany). HPLC grade methanol and acetonitrile were obtained from Merck, Germany. High-purity water (18 MΩ) was employed throughout (Wani *et al*., 2021). Saffron stigmas were placed in a Pyrex 100 × 50 mm glass dish and cut using a razor blade to "grind" the saffron as evenly as possible. The ground material was collected using a spatula and paintbrush into pre-weighed 20 mL scintillation vials. 50 mg of each saffron sample was weighed in a 25 mL volumetric flask for extraction.

To determine crocins and safranal in saffron, stock solutions (1 mg/ml) of crocin and safranal were prepared in methanol, and 10-30 µg of these compounds were used for the calibration curve to check the linearity. A calibration curve was prepared based on the external standard using final concentrations of 10, 20 and 30 µg concentrations of crocin and safranal in triplicate. Samples were extracted with 8 mL of degassed methanol, sonicated for 1 h, and then stored overnight. The whole process was carried out in the dark at room temperature. Samples were removed from darkness, sonicated for one more hour, and brought to volume with previously degassed methanol. Each extracted sample was filtered using a 1 mL Tuberculin syringe and a 0.20 µm filter tip into an HPLC vial for HPLC analysis.

HPLC measurements were performed on an Agilent 1100 HPLC with a G1314A UV detector at The Research Institute of Material Sciences, New Delhi (India). Chromatographic separation was performed on a Waters C18 250 mm×4.6 mm, 5 µm column at 35°C. The mobile phase was a binary gradient: distilled water with 0.1% (v/v) orthophosphoric acid (85 per cent) and acetonitrile. The linear gradient was 15 per cent

acetonitrile for 5 minutes. The flow rate was 1 mL/min, and the injection volume was 10 µL. Safranal was detected at 310 nm, and crocin was detected at 440 nm. Quantitative analyses were performed into account with respect to molecular coefficient absorbance for every peak acquired at the specific wavelength of maximum absorbance of the relevant compound(s) reported earlier. Concentrations of crocin and safranal were expressed in mg/g of saffron stigma (Alam *et al.*, 2016).

Statistical analysis: The statistical analysis for the Completely Random Design (CRD) was done by ANOVA using Opstat software (Sheoran *et al.*, 1998) (*http://14.139.232.166/opstat/*).

RESULTS AND DISCUSSION

Saffron colour and aroma quality depend on crocin and safranal concentration, respectively. Saffron obtained in the third year of cultivation from areas outside traditional saffron growing areas was compared for these quality parameters, and results are presented in Table 2 and Fig 2. The chromatographic conditions employed allowed quantification of crocin and safranal with retention times of 30 and 41 min, respectively **(Fig 3)**. These components were identified by comparison of their retention times as previously reported (Lonzano *et al.*, 1999; Caballero-Ortega *et al.*, 2007). There was a significant difference ($p \leq 0.05$) in crocin content among the experimental sites. Also, a significant difference ($p \leq 0.05$) was found for safranal content between the experimental sites. The average values for crocins ranged between 28.40 mg/g and 48.96 mg/g on a dry weight basis. The maximum crocin content was recorded at Mirgund (48.96 mg/g) and the minimum at Mir Udhampur 28.40 mg/g (Table 2). The highest safranal content (1.18 mg/g) was obtained in samples of Pampore (S_0), while the minimum (0.85 mg/g) was at Shiva Doda (S_{11}). Crocin and safranal content from sites of the Kashmir region was found on the higher side than the saffron from sites of the Jammu region (Table 2, Fig 2). Saffron from Pampore (S_0) is superior to the saffron from other locations in terms of crocin and safranal content taken together.

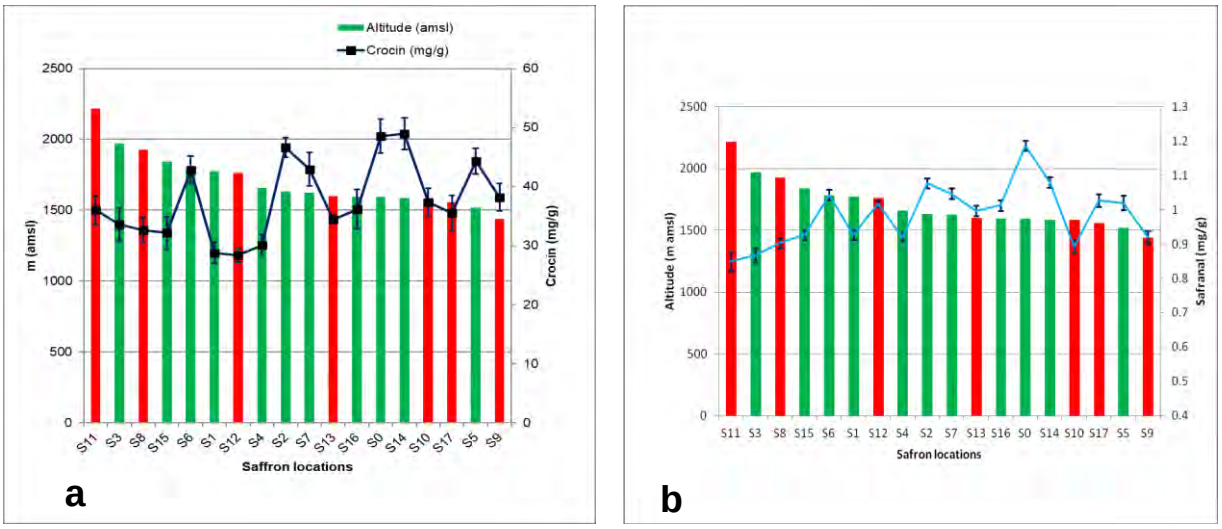

Fig. 2: Saffron quality obtained at different locations arranged in the decreasing order of altitude a) Crocin content (colour), b) Safranal content (aroma) ; (Red bars denote places in Jammu, and Green bars denote places in Kashmir)

The total content of crocin and safranal depends upon many factors, including storage of stigma and extraction method of apocarotenoids (Caballero-Ortega *et al.*, 2007). Hence the amount of these compounds reported by different workers vary. In the present study, the crocin content varied between 28.40-48.96 mg/g. TLC and HPLC analysis of Indian saffron samples obtained from the local market revealed that crocin content was 67.3 mg/g and 48 mg/g, respectively (Sujata *et al.,* 1992). HPLC assay of saffron samples from Spain, Iran and Tibet revealed a varied level of Crocin concentration in these samples and was found to be 9.00 mg/g, 2.90 mg/g and 2.80 mg/g dry weight, respectively (Li *et al.*, 1999). Crocin content of saffron samples obtained from the experimental farm of Sara Alghoniam Research Chair of Saudi Arabia ranged from 10.43-16.32 mg/g (Alam *et al.*, 2016). In contrast, crocin content from Iranian saffron samples was recorded as 69.3 mg/g (Farrokhi *et al.,* 2021).

Table 2: Quality of saffron stigma obtained at different locations using HPLC method

Location code	Location name	Altitude (m amsl)	Crocin (mg/g)	Safranal (mg/g)
S_0	Pampore (Control)	1593 m	48.53	1.18
S_1	Dooru, Anantnag (KVK)	1773 m	28.77	0.92
S_2	Malangpora, Pulwama (KVK)	1630 m	46.67	1.07
S_3	Balpora, Shopian (KVK)	1970 m	33.58	0.86
S_4	Kulangam, Kupwara (KVK)	1658 m	30.12	0.92
S_5	Haran, Budgam (KVK)	1520 m	44.30	1.02
S_6	Benhama, Ganderbal (KVK)	1784 m	42.84	1.04
S_7	Shalimar, Srinagar (FoH)	1625 m	42.92	1.04
S_8	Budhal, Rajouri	1926 m	32.62	0.90
S_9	Jalian Mandi, Poonch	1440 m	38.17	0.92
S_{10}	Mahore, Reasi	1582 m	37.35	0.89
S_{11}	Shiva, Doda	2215 m	36.01	0.85
S_{12}	Mir, Udhampur	1761 m	28.40	1.01
S_{13}	Gandhri, Ramban	1600 m	34.43	0.99
S_{14}	Mirgund, Baramulla (TSRI)	1584 m	48.96	1.08
S_{15}	Pombay, Kulgam (KVK)	1841 m	32.15	0.92
S_{16}	Shuhama, Ganderbal (KVK)	1595 m	36.15	1.01
S_{17}	Pochal, Kishtwar	1557 m	35.49	1.02
C.D ($p \leq 0.05$)			**6.846**	**0.048**

In the present study, HPLC analysis revealed that safranal ranged between 0.85-1.18 mg/g. In an earlier study, HPLC analysis of Indian saffron samples obtained from the local market revealed that safranal content was 4 mg/g (Sujata *et al.,* 1992). In another study, the HPLC quantitative analysis of saffron from 10 different saffron countries viz. Greece, India, New Zealand, Spain, France, Azerbaijan, Italy, Turkey, Iran and China

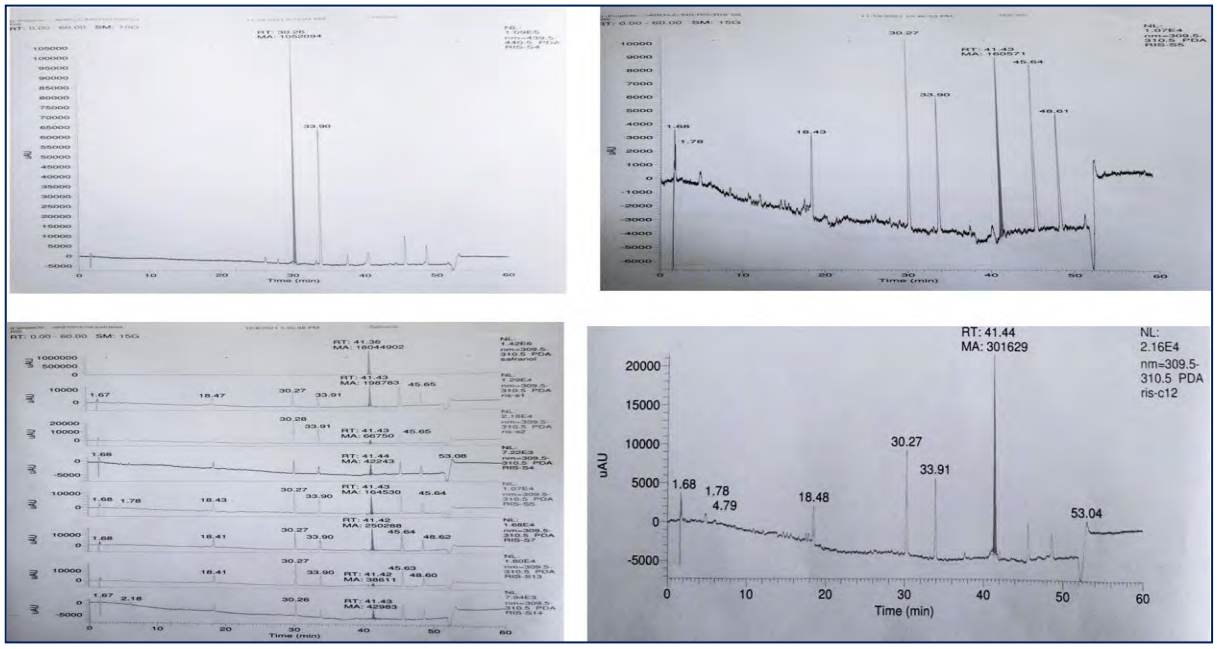

Fig. 3: Chromatograms of crocin and safranal with retention time

revealed that safranal concentration among these samples varied a lot and was found to be 1.29, 1.24, 0.47, 0.88, 0.81, 0.98, 0.53, 0.84, 0.65, 0.22 mg/g, respectively (Caballero-Ortega *et al.,* 2007). The range of safranal content 5.19-5.21 mg/g has been reported from saffron samples obtained from the farm of Sara Alghoniam Research Chair of Saudi Arabia (Alam *et al*., 2016). In comparison, a safranal content of 15.8 % was recorded from Iranian saffron samples (Farrokhi *et al.,* 2021).

The variation in altitude among these sites ranged between 1440-2215 m amsl, while the crocin content varied between 28.40-48.96 mg/g and safranal ranged between 0.85-1.18 mg/g. This shows that the location of the experimental site significantly affected the saffron quality. However, many factors may contribute to these differences, as there was no direct correlation between altitude and the concentration of these two compounds. The factors that could directly or indirectly influence the saffron quality include the mineral content of the soil and its microbiome, temperature and rainfall during critical growth and development phases, and crop management practices. At the genetic level, all these factors can lead to the modulation of apocarotenoid gene expression, thereby influencing the content of bioactive compounds in saffron.

The crocin and safranal content depends upon many factors, including the developmental stage of stigma, storage of stigma, processing and extraction method etc., and hence there are so many variations between studies on saffron quality. The amount of these compounds varies from sample to sample and between different workers. The results obtained from the 18 locations across J&K clearly show a significant difference in crocin and safranal contents between these locations. In an earlier study on saffron cultivation in the different locations of the temperate region of Kashmir, pH and electric conductivity may have been responsible for variations in the chemical characteristics of saffron (Muzaffar et al., 2018). The best quality saffron with high concentrations of crocin and safranal was obtained from traditional (Pampore) and was closely followed in quality by Mirgund (Baramulla), a non-traditional site. This offers an opportunity to explore the possibility of saffron crop expansion outside traditional areas.

CONCLUSION

There is an untapped potential to introduce saffron in the marginal areas of the trans-Himalayan region of Jehlum and Chenab valleys of Jammu and Kashmir (India). The present study was conducted across 16 districts of J&K to determine saffron quality from non-traditional areas and find promising sites for saffron introduction. Districts are administrative units with similar or variable ecological zones among them, and even the same district could have variations in saffron quality. The factors that may affect the saffron quality include the edaphic (soil microbiome, mineral and organic carbon content), weather (average temperature, rainfall, extreme weather events), and crop management practices. All these factors need to be studied in detail as these can modulate apocarotenoid gene expression at the genetic level, thereby influencing the profile of bioactive compounds in saffron. Pampore (Control), Malangpora (Pulwama), Haran (Budgam), Benhama (Ganderbal), Shalimar (Srinagar), Mirgund (Baramulla) were statistically at par with respect to crocin content, which is responsible for colour in saffron. Among these six sites, the only traditional site where saffron has been cultivated since ages is the famous area of Pampore, while at all the other ‘sites’, saffron was cultivated for the first time. For safranal content (aroma) Pampore (Control) was the best, and was closely followed by sites in non-traditional areas of Mirgund (Baramulla), Malangpora (Pulwama), Benhama (Ganderbal) and Shalimar (Srinagar). Introducing saffron in such areas would help fulfil the domestic saffron demand and contribute to earning foreign exchange through its export. It can lead to overall growth in the saffron-based green-economy and enhance the individual income of saffron farmers.

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