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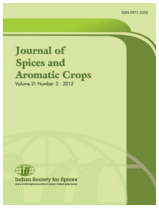

Variability in stigma length and apocarotenoid content in *Crocus sativus* L. selections of Kashmir

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Abstract

Identification of high yielding selections using the existing gene pool of saffron shows promise and potential for improving the productivity of this crop. The present study was conducted at Srinagar (Jammu & Kashmir) with 31 saffron selections. Variations in respect to stigma length was observed. Apocarotenoid content was correlated with stigma length of saffron. Stigma size vis-a-vis quality evaluation confirmed that saffron of Kashmir is of intrinsically high quality with respect to colouring, aroma and taste. Variability in stigma characteristics observed in saffron selections can be utilized for saffron crop improvement.

Keywords: *Crocus sativus*, crocin, picrocrocin, saffron, safranal

The genus *Crocus* belong to Iridaceae family includes native species from Europe, North Africa and temperate Asia and is especially well represented in arid countries of south-eastern Europe and Western and Central Asia. Among the 85 species of genus *Crocus*, *C. sativus* L. (Saffron) is the most fascinating and intriguing species (Fernández 2004). *C. sativus* is an autumnal flowering geophyte with corms that are covered, dormant during summer, sprouting in autumn, and producing 1-4 flowers in a cataphyll. Saffron is histoanthus and flowers are formed prior to leaves. The nutrition required for flowers is supplied by underground corms. The flower has an underground ovary, a style 9–10 cm long, dividing at the top in three red trumpet-like stigmas (usually 2.5 cm long) that once dried form the commercial spice saffron. Stigma length variability has been observed in Kashmiri saffron by Nehvi *et al*. (2005). Many methods of saffron component analysis have been described by Tarantilis *et al*. (1995) and the method of saffron quality characterization currently recommended by the International Standardization Organization is UV–vis spectrophotometry (ISO/TS 3632, 2003). The quality of saffron is certified in the international trade market following the ISO 3632 Normative since 1993. The most important parameter is colouring strength, calculated from UV-Vis measurements at 440 nm in aqueous extracts of this spice. Such measurements are related with the total crocins content. Other important components are picrocrocin and safranal, responsible for flavor and aroma respectively.

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The amounts of these main compounds are used to express the quality of saffron. The higher the amounts of these compounds in saffron, means higher quality of saffron. According to ISO 3632 classification, best quality saffron belongs to category I, which means direct reading of the absorbance at about 440 nm, 330 nm and 257 nm for crocin, saffranal and picrocrocin is greater than 190, 20 and 70 respectively as reported by Tarvand (2005). Stigma length will have direct impact on apocarotenoid content and yield of saffron. Hence variation in stigma length across different selections of saffron has to be correlated with apocarotenoid values. In this study, the stigma length which is an important quantity parameter of different saffron selections was correlated with apocarotenoid values of saffron extracts.

The experiment was carried out at Central Institute of Temperate Horticulture, Srinagar during 2009–11. Standards of crocin, safranal and picrocrocin were obtained from Sigma Aldrich. A total of 31 saffron selections were used in the study which were obtained from Central Institute of Temperate Horticulture, Srinagar, Pampore and Budgam districts of Kashmir. Full-bloomed flowers were picked up from CITH farm during mid-October to mid-November for three successive years (2009–2011). The three-lobed stigmas along with the styles were separated manually just after picking and dried. Samples were dried in saffron drier (Developed by SKUAST-K, Srinagar) at 60ºC. Dried samples were ground in pestle and mortar and were passed through a 0.5 mm mesh and stored in the dark at 4ºC until they were used. A 100 mg mass of dried stigma was extracted with 5 mL of cold 50% (v/v) ethanol in a pestle and mortar, it was then transferred to a screw-capped 50 mL tube and a total volume of 20 mL 50% (v/v) ethanol was added to it. Tubes were sonicated for 20 min on ice and then centrifuged at 4000 rpm for 15 min and washed twice with 5 mL each of the 50% (v/v) ethanol. The supernatant was used for analysis by spectrophotometric procedures. Saffron samples were analyzed according to the ISO 3632 trade standard (ISO/TS 3632, 2003). This method allows the determination of the main characteristics of saffron related with picrocrocin, safranal and crocins content. Higher amount of these components means higher quality of saffron. According to ISO, picrocrocin, safranal and crocins are expressed as direct reading of the absorbance of 1.0% aqueous solution of dried saffron at 257, 330 and 440 respectively. The supernatant (1 mL) was diluted to 5 mL with 50% (v/v) ethanol for analysis using Nanodrop (Make Thermo). A standard curve was prepared by measuring the absorption of crocin, saffranal and picrocrocin at 440 nm, 330 nm and 257 nm respectively. Sample supernatants were diluted 100 times and readings were taken at 440 nm, 330 nm and 257 nm for crocin, saffranal and picrocrocin respectively. Measurements of E1% of aqueous saffron extract at 440, 330 and 257 nm, respectively, were done using a 1 cm pathway quartz cell. Results are obtained by direct reading of the absorbance, D, at three wavelengths, as follows:

$$E1\%\ 1\ cm = [D \times 10000]/ [M \times (100-H)]$$

Where D is the specific absorbance; m is the mass of the saffron sample in grams; H is the moisture and volatile content of the sample, expressed as a mass fraction. Moisture and volatile contents were identified by using powdered saffron stigmas. The samples were ground with a pestle and mortar and passed through a 0.5 mm mesh. After weighing, the powdered samples were introduced uncovered in an oven set at 103ºC for16 h. The moisture and volatile matter content are expressed as a percentage of the initial sample using the following relation: [(initial mass-constant mass)/initial mass] × 100. Observations on stigma characteristics were recorded in five replications. The data was analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using SPSS for windows (v. 15. SPSS Inc USA). Two tailored Pearson correlation was done between stigma length and apocarotenoid values through SPSS for windows (v. 15. SPSS Inc USA).

Average stigma length varied from 2.86 cm in

selection PAM-S-116 to 4.84 cm in CITH-S-107 (Table 1), which is higher than reported earlier by Caiola (2004) and Nehvi *et al*. (2007). Nehvi *et al.* (2007) reported stigma length range of 2.41-3.87 in 438 random selections of saffron from Kashmir. Apocarotenoid values showed significant variation across 31 saffron selections. Values for crocin content varied from 151 in selection PAM-S-116 to 195 in CITH-S-107. Stigma length of saffron selections was found to be positively correlated with crocin values (0.255). Saffranal values ranged from 23 in PAM-S-101 to 44 in CITH-S-118. Sigma length showed negative correlation (-0.19) with saffranal values. Safranal is formed during the handling and storage of saffron, and also by subsequent chemical or enzymatic dehydration of the picrocrocin as reported by Ibora *et al.* (1992). Picrocrocin values ranged from 53 in PAM-S-111 to 87 in CITH-S-115 and was found to be positively correlated (0.257) with stigma length of saffron selections. Direct readings $E^{1\%}$ of extracted saffron samples at 440 nm were 150-190 for 30 samples which thus fell in category II as per ISO 3632-2, clause-13 and > 190 or one sample which fell in category I ISO 3632-2. Similarly, direct readings $E^{1\%}$ of extracted saffron samples at 257 nm were >70 for 15 samples, 55-70 for 13 samples and 40-55 for three samples falling in category I, II and III respectively according to ISO classification. Direct reading of saffron samples at 330 nm ranged from 23-44 which is between 20-50. As indicated by Alonso *et al*. (2001), these results show that the direct reading of safranal at 330 nm which is a criteria for safranal content (according to IS0 3632) fell within the optimum ranges.

Table 1. Variability in stigma length of different saffron selections

Selections	Stigma length (cm)
CITH-S-125	3.74^{k}
CITH-S-123	4.38^{n}
CITH-S-124	3.86^{kl}
CITH-S-122	3.98^{l}
CITH-S-12	3.44^{j}
CITH-S-121	4.14^{m}
CITH-S-107	4.84^{o}
CITH-S-120	3.86^{kl}
CITH-S-104	3.72^{k}
CITH-S-117	3.3^{hij}
CITH-S-112	3^{abcde}
CITH-S-113	3.16^{efgh}
CITH-S-119	2.98^{abcd}
CITH-S-118	3.22^{ghi}
CITH-S-10	2.9^{ab}
CITH-S-103	3.04^{bcdef}
CITH-S-43	3.16^{efgh}
CITH-S-114	3.3^{hij}
CITH-S-115	3.2^{fghi}
CITH-S-105	3.08^{cdefg}
PAM-S-106	3.34^{ij}
PAM-S-102	3.14^{defgh}
PAM-S-108	3.4^{j}
PAM-S-11	3.3^{hij}
PAM-S-116	2.86^{a}
PAM-S-13	3.42^{j}
PAM-S-101	3.7^{k}
PAM-S-3	3.3^{hij}
PAM-S-111	3.12^{defg}
BUD-S-110	2.92^{abc}
BUD-S-76	3.2^{fghi}

Means followed by the same letter within the columns are not significantly different (P<0.05) using DMRT.

The evaluation of saffron selections was done according to the limit set by the ISO 3632/TS normative (ISO/TS 3632-1/2, 2003). The major focus of this measure is to establish whether the product meets the ISO standard parameters, to get an indication of the range of quality being produced under Kashmir conditions and to have a comparison between the selections. Results showed that as high as 50% of the samples had high picrocrocin values and belonged to category I, all the samples had higher aroma strength falling under optimal range to meet ISO standard parameters for good quality (Table 2). But with respect to colour development only one selection fell under category I and rest of the 30 selections fell under category II. This may be due to poor yield of crocin through this method of extraction. Carmona *et al.* (2004) reported that extraction

Table 2. Apocarotenoid variability in different saffron selections

Name of the Selection	Crocin	Safranal	Picrocrocin
CITH-S-125	188^{k}	23.2^{a}	79^{fg}
CITH-S-123	161de	23.6^{ab}	68^{d}
CITH-S-124	181^{j}	36.2^{efghi}	78^{f}
CITH-S-122	159^{bcd}	42.8^{hi}	77^{ef}
CITH-S-12	158^{bc}	33.6^{defg}	79^{f}
CITH-S-121	161^{cde}	40.8^{ghi}	77^{ef}
CITH-S-107	195^{l}	28^{abcd}	83^{gh}
CITH-S-120	161^{cde}	38.8^{fghi}	73^{e}
CITH-S-104	161de	37.6^{fghi}	78^{f}
CITH-S-117	171^{fg}	32.4^{cdef}	77^{ef}
CITH-S-112	173^{g}	32.4^{cdef}	75^{ef}
CITH-S-113	169^{f}	42.6^{hi}	65^{cd}
CITH-S-119	181^{j}	36.2^{efghi}	62^{bc}
CITH-S-118	159^{bcd}	44^{i}	56^{a}
CITH-S-10	169^{f}	39^{fghi}	62^{bc}
CITH-S-103	161^{cde}	31.4^{cdef}	66^{cd}
CITH-S-43	179^{hij}	29.2^{abcde}	69^{d}
CITH-S-114	179^{ij}	23.6^{ab}	85^{h}
CITH-S-115	160^{bcde}	37.2^{efghi}	87^{h}
CITH-S-105	176^{h}	29.2^{abcde}	84^{h}
PAM-S-106	179^{hij}	24.8^{abc}	53^{a}
PAM-S-102	171^{fg}	43.4^{hi}	65^{cd}
PAM-S-108	181^{j}	43.8^{i}	53^{a}
PAM-S-11	157^{b}	35.6^{defgh}	61^{b}
PAM-S-116	151^{a}	36.6^{efghi}	61^{b}
PAM-S-13	172^{fg}	31.2^{bcdef}	75^{ef}
PAM-S-101	186^{k}	23^{a}	83^{gh}
PAM-S-3	177^{hi}	33.4^{defg}	62^{bc}
PAM-S-111	163^{e}	32^{cdef}	53^{a}
BUD-S-110	158^{bc}	34.2^{defg}	60^{b}
BUD-S-76	162de	34.6^{defg}	63^{bc}

Means followed by the same letter within the columns are not significantly different ($P<0.05$) using DMRT.

procedure, method of detection and type of solvent used in extraction process had effects on total crocin content. Hence, further refinement is needed to recover maximum crocin pigment during the extraction process. Quality evaluation confirmed that saffron of Kashmir is of intrinsically high quality with respect to colouring, aroma and taste. The results are in general agreement with earlier reports of Nehvi *et al*. (2005 & 2007). Present study showed that there is heterogeneity among the saffron selections with respect to stigma length and apocarotenoid content. Thus, utilization of heterogeneity in the natural population which is due to genetic and environmental factors offers tremendous scope for saffron improvement.

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