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Morpho-Biochemical Studies of Saffron Under Cold Arid Regions of Ladakh

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Abstract

The present study was under taken at six locations in Cold Arid regions of Ladakh under open field and protected conditions. The results of the study revealed that shading caused by less sunshine hours in the cold arid zone of Ladakh probably has balanced soil temperature and has improved the growth of saffron which has influenced the final produce of the crop. Further, quality of the produce (stigma) viz., crocin, picrocrocin and safranal contents was recorded higher under protected conditions as compared to the open field condition particularly under cold arid regions. This study suggests that differences in altitude and climatic factors among regions can significantly affect saffron yield and quality.

Keywords: Open field; Protected conditions; Yield attributes; Quality; Saffron; Cold arid region

Introduction

Saffron (*Crocus sativus* L.) is an important spice, known for its aroma, colour and medicinal properties and is regarded as the costliest spice in the world which includes around 80 species mainly distributed in the Mediterranean area and in the south-east of Asia. Despite its wide popularity which has enabled all saffron producing countries to increase production during the last 3-4 decades, there has been a decrease in saffron production in almost all the countries. One of the major causes for such a decline in the productivity of saffron is the use of low-quality planting material. Corm accounts for single most costly input in saffron cultivation. Saffron is a sterile plant that has no seeds and propagates vegetatively by means of a corm. Therefore, selection of corms for propagation intention is an important factor in saffron production. Yield and quality of stigma and corms are affected by corm size. The determination of most suitable corm size would contribute to application of cultivation practices and ultimately yield and quality. An attempt to modernize saffron cultivation will therefore require efficient mass production of corms. Saffron research at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, India have also confirmed importance of corm size as one of the important components in improving saffron productivity. Thus, it is realized that the study of behaviour of saffron corms for daughter corm production is of paramount importance for economizing the commercial scale corm production and making corms available for area expansion. Therefore, the selection of quality corm production are crucial factors for saffron cultivation [1-3].

Several scientific studies have reported that saffron and its chemical components are potential anti-ulcer agents, they improve digestion, they play a role as anticancerogeni, they are used as anti-depressant in the traditional medicine. The main compounds that contribute to the sensory profile of saffron are crocin (mono-glycosyl or di-glycosyl polyene esters), picrocrocin (monoterpene glycoside precursor of safranal) and safranal (aldehyde). Guidelines for the analyses of these major bioactive compounds have been established by the International Standards Organization. They have defined procedures to determine these compounds by spectrophotometric analyses and have established the limits by which saffron quality is classified. According to ISO 3632, picrocrocin, safranal and crocin express the flavour or bitterness, the aroma and the colouring respectively. These values are defined as direct reading of the absorbance of a 1% aqueous solution of dried saffron at 257, 330 and 440 nm using a 1 cm pathway quartz cell.

Corm production/ multiplication of saffron under both open and protected conditions has been taken up only in recent years on experimental basis and the returns realized were more or less equal to the returns obtained from the economic yield (stigmas) of the saffron**.** The aim of this investigation was to evaluate the morpho-biochemical characteristics of saffron under open field and protected conditions in the cold arid regions of Ladakh viz., Kargil and Zanaskar [4].

Material and Methods

The experiment was conducted during the year 2018-2021 at six cold-arid regions with different altitudinal zones in two districts of Ladakh (Kargil and Zanaskar). Kargil is 2,676 metres (8,780 feet) above sea level and is situated along the banks of the Suru River (Indus) with an average temperature of 8.6^{O}C. Zanskar Range is part of the Tethys Himalaya, an approximately 100-km-wide synclinorium having an average height of about 6,000 m (19,700 ft) with an average temperature of 10 ^{O}C. Four locations were selected from Kargil region viz., Sankoo, Batalik, Trespone and Shergol. Two were selected in Zanaskar viz., Panikhar and Nerok. The trials were conducted under open field and protected conditions at all the six locations (Figure 1).

Field experiments

Corms of 3.0–3.5 cm diameter and 11.0–15.0 g weight were sown under field conditions. Corms were placed in raised beds, 80 cm wide and 30 cm high from soil level, with 4 rows of corms in each bed at 10 cm depth. Row to row distance was 20 cm and 5 or 6.7 cm within rows. A randomized block design (RCBD) was adopted with three replications. In both environments, soil was fallow in the previous year and no fertilizer and irrigation was applied. Weed management

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Figure 1: Foliage stage of the crop under open field and protected conditions.

was carried out by hand throughout the experiment. High Density Production System Module, Irrigation module, Nutrient Management Module etc. were used for raising a good crop. Corms were also sown under protected environmental conditions. Six low cost Poly-houses were constructed out of which 4 were in District Kargil and 2 in District Zanaskar for corm multiplication under protected conditions.

The data on the length of Style (cm), Pistil length (cm), Fresh weight of pistil (mg) and Dry weight of pistil (mg) was recorded. Whole flowers were manually picked daily early in the morning in the first hours after sunrise, before the flower had completely opened. Immediately after flower picking, fresh stigmas were separated by hand from the remaining flower and were vaccum dried as per the standard procedure [5].

Apocarotenoid quantification

Extraction procedure: The saffron extracts were prepared according to ISO 3632 (2011). Methanolic extract was prepared using 20 mg of saffron stigmas grounded in 5 ml of methanol and 5 ml of distilled water (2 mg/ml) for 2 hours at room temperature. In order to separate the residual fraction of the extracts, the samples were centrifuged at 4000 rpm for 10 minutes. The solution was filtered through a 0.45 μm pore sized hydrophilic polytetrafluoroethylene (PTFE) filter and stored at -20^{o}C until used [6].

UV-Vis spectrometry: In order to perform UV-vis spectrometry analysis according to ISO 3632 (2011), the extract was diluted to 1:10 (v/v) and then scanned at a wavelength of 440, 330, and 257 nm using a UV 160 A, UV-visible recording spectrophotometer in a 1 cm pathway quartz cell.

The data regarding various characters were statistically analyzed using SPSS software. Statistical differences among treatment means were tested by F-test and when the F-test was found to be significant, critical difference (C.D) at 5% significance level was calculated [7].

Results and Discussion

Morphological and productive traits

Growth parameters viz., style length and pistil lengths were significantly affected by different geographical locations. A significantly higher length of style was recorded in L_1 (Panikhar) (2.40 cm) followed by L_6, L_3, L_5 and L_4. Lowest length was recorded in L_2 (Sankoo) (1.80cm). Under protected conditions, style length was observed highest at Trespone, Kargil (2.43cm) and lowest (2.07cm) at Nerok, Zanaskar location. Sand-particles usually cause more pores, which improves the permeability of the soil (Banihabib and Iranpour, 2012). This increases the root growth, improves the production and development of leaves. A significantly higher length of pistil was recorded in L_6 (Shergol) (5.7cm) followed by L_1, L_4, L_5, L_3 while the least pistil length was observed at L_2 (Sankoo) location (4.7cm). In their study reported efficient cultivation of saffron at the highest altitude, which is most desirable for corm production, suggesting that elevation can play a crucial role. Pistil length was found to be higher in protected environment as against the open field conditions being largest at Trespone location (6.43cm) and smallest (5.30cm) at Nerok location. The development of corms is directly dependent on shoots or leaves at appropriate environmental conditions, including temperature, altitude and soil texture for saffron growth [8].

During the present study flower yield was higher at an altitude of 2,670m (Batalik, Kargil, Ladakh) as compared to other places. It might be because of the favourable environmental conditions, viz., average temperature, total rainfall and relative humidity. Fresh pistil yield was significantly higher in location L_3 (Batalik) (33.00 mg) which was followed by L_4 (32.00 mg), L1 (28.73 mg), L6 (27.67 mg) and L2 (26.67 mg) and lowest fresh pistil yield was recorded at L_5 (Nerok) (24.67 mg) location. Under protected conditions fresh pistil weight was found to be much higher as compared to open field conditions. Shergol which is situated in Kargil showed highest fresh yield of 40.07mg and lowest was observed in Nerok, Zanaskar (33.0 mg). These results indicates that saffron preforms better under protected conditions with adequate irrigation and temperature control than open field conditions. Rainfall and temperature are the crucial climatic factors controlling the growth and flowering in *Crocus species*. Dry stigma yield was significantly affected by different altitudinal variations. In location L_3 (Batalik, Kargil), significantly higher dry stigma yield was observed compared with other different geographical locations. The dry stigma weight at L_3 location was 6.53 mg which was significantly higher over other locations. In Greece the average yield ranges from 4.0–7.0 kg/ha, Italy from 3.4 to 10.0 kg/ha, in Morocco 2.0–2.5 kg/ha; in Spain 2.5–6.0 kg/ha and in Iran 3.0–5.0 kg/ha. In our investigation, the average dry pistil yield under protected conditions varied from 7.97mg (Shergol)-6.37mg (Nerok). The geographical location of Batalik, Kargil (L_3) significantly recorded higher dry stigma yield as compared to other sites, however, the dry yield of stigma behaved statistically at par with Sankoo, Trespone and Shergol which were the other sites in Kargil. The high input of organic matter and low decomposition processes is carried out at high altitudes, as temperature and temporal water saturation contributed to increased yield. The lowest dry pistil yield was recorded in Nerok, Zanaskar as the altitude was relatively low and rainfall was higher as compared with other locations. It might also be related to organic carbon, available nitrogen, phosphorous, potassium, soil texture and other external environmental factors. Specifically, the climatic conditions of these locations were desirable for the production of the saffron corm and other vegetative characteristics. Previous studies reported a 70% increase in saffron flowering when planted in soils with lighter consistency however, an 18% increase was reported in saffron stigma yield when grown in sandy soil as compared with the heavy soil [9].

Quality Analysis

Results of picrocrocin (flavour strength), safranal (aroma strength) and crocin (colouring strength) amount (expressed as mg/gm) of each location are presented. Saffron quality in different regions of

Ladakh province showed that the amount of crocin, picrocrocin and safranal varied slightly among different regions, but there was a visible difference for these compounds between samples collected from open field and those collected from protected environment. The highest amount of crocin under open field conditions (43.21 mg/gm) as well as protected conditions (45.42mg/gm) was obtained from the stigma samples that were collected from Batalik, Kargil. The differences between the amount of crocin in Kargil and Zanaskar districts were not significant and all of them were in the same group. Although, there was no significant difference for the amount of crocin in different districts, but the difference was observed between the samples from open field and protected conditions. Panikhar location showed lowest crocin content 40.12mg/gm under open field conditions while under protected conditions lowest crocin content lowest crocin content (41.13mg/gm) was observed at L_5 (Nerok) location. Amount of safranal was recorded highest from Shergol, Kargil (0.25mg/gm) and lowest at Sankoo location (0.21mg/gm). Under protected environment, this compound ranged from 0.27mg/gm to 0.24mg/gm which is again on the higher side as compared to open field conditions. Picrocrocin ranged between 1.08mg/gm (Trespone, Kargil) to 0.87 mg/gm (Sankoo, Kargil). Highest amount of picrocrocin (1.15mg/gm) was observed at L_4 location while lowest (1.12 mg/gm) was observed at L_2 location. Factors affecting the accretion of compounds in plants used as medicinal and pharmaceutical raw materials and food additives are dependent on altitude, temperature, soil type, irrigation cycles, plant quality and harvest times of saffron crop. Increased crocin and picrocrocin at higher altitudes might be due to mean air temperature, solar radiation and soil characteristics which affects the accumulation of marker compounds in plants. The drying process also influences the product's quality Previous studies indicated that with the increase in altitude, the crocin content increased and phenolics / flavonoids were also increased at higher altitude locations as compared with lower altitudes. Reported that crocins and picrocrocin values decreases naturally in the stigmas cell during drying, storage and extraction process [10-12].

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