The Growth of Saffron (Crocus sativus L.) in Aeroponics and Hydroponics

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ABSTRACT: The development of saffron (Crocus sativus L.) plants and the production of commercial saffron and saffron constituents were compared in three culture systems: aeroponics, hydroponics, and soil. On a dry weight, but not fresh weight basis, corm growth was increased in aeroponics and hydroponics as compared with growth in soil. Root length in aeroponics and hydroponics was reduced as compared with root length in soil, but shoot development was not significantly affected. Flowering was poor in all three culture systems, probably due to the small-sized (2.6 cm) bulbs used in propagating the plants. The production of stigmas and the concentration of the main constituents of saffron in the stigmas was similar in all three culture systems, suggesting saffron bulbs grown aeroponically and hydroponically may be used as a practical and renewable source of pharmacological compounds extracted from saffron. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-6958. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]

KEYWORDS. Crocetin, crocin, growth, picorocin, plant culture, safranol

INTRODUCTION

Cultivation of saffron, Crocus sativus L. (Iridaceae), is labor intensive and time consuming, factors that contribute greatly to the price of
the spice (12). Propagation of the species is via daughter corms from
the parent as the plant is non-fertile (12). Pathogenic fungi and viruses
transferred from planting to planting by infected corms frequently
attack the plant (14,20). Harvesting the large red stigmas that after
drying constitute commercial saffron is difficult and time consuming
(18). Only 6 kilograms of saffron, about 900,000 flowers, are har­
vested per hectare of culture (26).

The difficulties associated with production have made saffron the
most expensive spice in the world. In the U.S. market that uses saffron
for food coloring and flavoring, the price of the spice has reached
$2,000/kg. Yet, demand for saffron and saffron extracts is increasing
as new applications in pharmacology have emerged in recent years
(19,22). Natural constituents in saffron stigmas include crocin (the
main constituent), crocetin (which with crocin is responsible for the
intense red-orange color), picrocrocin (the bitter principle) and safra­
nal (the most abundant volatile oil in saffron) (7,20,24).

Soilless plant culture using hydroponics and aeroponics, has per­
mitted the growth of several plants under conditions in which normal
cultivation is difficult or impossible (5,21,27). By using hydroponics
or aeroponics, the growth environment and nutrition of the plants can
be carefully controlled, resulting in higher yield and generally better
quality product (10,13,27,28). In hydroponics, the plant roots are
maintained in either a static, continuously aerated nutrient solution, or
a discontinuously flowing nutrient liquid solution (2). In aeroponics,
water and minerals are supplied to the plant via a mist that deposits
fine droplets of nutrient solution on the roots (5,27,30). Such soilless
culture systems could perhaps offer an alternative to the current field
culture of saffron production.

In this study, the growth of saffron bulbs in hydroponic and aero­
ponic systems were evaluated for the production of flowers with high
quality stigmas.

MATERIALS AND METHODS

Plant material. Corms of saffron (Crocus sativus L.) (averaging 2.6
cm in diameter, and 5.7 g fresh weight) purchased from Nichols Gar­
den Nursery (Albany, OR) in early September and grown in nutrient
culture (half-strength Hoagland medium in hydroponic and aeroponic
systems) (9) and soil were used in these experiments. [Saffron bulbs
bloom in the fall and can only be obtained in September, although storage for up to two months at 4°C (personal communication with Nichols Garden Nursery) or at 15°C under dry conditions (15) is possible. All plants were grown in an environmentally controlled culture room (temperature maintained at 17°C ± 2) (15). A photoperiod of 11 h light/13 h dark was established in the culture room to mimic natural light conditions in Spain and Israel where saffron is commercially produced. Incident light on the saffron was 150 ± 20 µmol m⁻² s⁻¹ from cool white fluorescent bulbs at corm tops.

Although the experimental nutrient cultures were not sterile, the nutrient medium was prepared in a four liter-flask and autoclaved at 121°C for 20 min before use to minimize the initial microbial load. Medium pH was adjusted to 6.0 to 6.25 before autoclaving and the electroconductivity (EC) was 1100 EC (µS/cm). The medium was replaced when the EC dropped below 1000 EC, every three or four days. For soilless cultures, the saffron corms were planted in two inch mesh plastic pots (CropKing, Inc., Seville, OH) filled with an inert chemical substrate (Leca stone, Einfach-Grun Hydroton, Germany).

**Experimental.** The hydroponic culture system used a nutrient film technology design (NFT) consisting of four channels, one catchment pipe, four inlet flow pipes, and a catchment tank (4 liter flask). The four channels were made of white PVC pipes (Worm's Way, Worcester, MA), 61.5 cm long and 7.5 cm wide, with equally spaced holes for two inch mesh pots. The catchment pipe was identical to the channels. The nutrient solution, stored in the catchment tank, was continuously aerated using two Whisper 200 aquarium pumps connected together in series. The solution was pumped (for a flow rate of 80 ml/h) through Norprene® black tubing (1/8 inch ID), using a Masterflex® multichannel pump, to the higher end of the channels (channels were set on a slight slope for a gravity feed) and discharged into the catchment pipe through 1/2 inch diameter PVC pipe adapters (Home Depot, Shrewsbury, MA) to conduct the nutrient solution back to the catchment tank. Flow rate was based on preliminary experiments done with onion sets.

The aeroponic culture system was based on an aeroponic nutrient mist bioreactor (NMB) modified from that described by Chatterjee et al. (4). The growth chamber, set on top of the mist bioreactor, was fabricated from a 2.27 liter Servin' Saver (Rubbermaid) container (20.5 cm diameter by 8 cm height) and the container cover (eight holes were cut in the cover to hold two inch mesh plastic pots). A nutrient
solution mist cycle of one min on and one min off with an air flow rate of six liter/min from the bioreactor to the growth chamber was established, corresponding to 16.4 ml/h of solution delivered to the growth chamber. A coalescer (Nalgene, Rochester, NY) was used to release excess pressure in the growth chamber while retaining the medium (4).

Corms in soil culture, used as a control for comparison with corms grown in the aeroponic and the hydroponic systems, were planted in a soil mix of 50 percent bulb planting soil (0-0.1-0 N-P-K; Scotts, Marysville, OH), and 50 percent general potting soil (Promix BX; Premier Horticulture Inc., Red Hill, PA). The corms in soil were watered with 250 to 300 ml of water every three days to maintain vigorous growth.

All corms were harvested six weeks after the beginning of an experiment. Flowers were picked at anthesis, six to seven days after appearance. Experiments were replicated seven times with eight plants per replicate.

Growth analysis. Growth rates of shoots and roots, initial and final bulb weight, productivity (size and weight of stigmata), and blooming date were measured in all plant growth systems. The dry weight of the bulbs was obtained after drying harvested bulbs at 55°C in a Lab-Line oven (Clinical Scientific Equipment Co.) for one week (18).

Saffron extraction and analysis. To determine the pigment content, the saffron (stigmas) were extracted in the dark at 4°C (to minimize constituent loss) with ethanol using a previously described technique (23). The dry stigmas (10 mg) were ground in a micro-homogenizer (Fisher-Scientific, Pittsburgh, PA) with 0.5 ml of chilled 80 percent ethanol (prepared from high-purity ethyl alcohol). After 5 min, the mixture of stigmas and ethanol was transferred into a 15 ml centrifuge tube and centrifuged at 6000 \( \times g \) for 10 min. The supernatant was decanted into a 1.5 ml Eppendorf tube, the pellet was washed two more times with 0.5 ml of 80 percent ethanol, and the washes added to the supernatant in the Eppendorf tube.

Crocin, crocetin and picrocrocin were identified by thin layer chromatography (TLC). The TLC was done on plastic backed plates, 20 cm \( \times \) 20 cm, of silica gel 60 F\(_{254}\), 0.2 mm thick (EM Separations, Gibbstown, NJ) activated before use by heating at 100°C for 1 h. Aliquots of the ethanol extract (50 µl) were loaded on the plates for the chromatography and the plates then inserted into a chromatography chamber that had been saturated overnight with the mobile solvent.
phase (1-butanol:acetic acid:water, 4:1:1, v:v:v). TLC separation was
done at room temperature in the dark for at least 6 h. Characterization
of the volatile safranal component was not done as this step required
further separation and development with 2-4, dinitrophenylhydrazine,
a solvent that we were unable to obtain commercially.

Commercial saffron and saffron powder, used as standards, were
purchased, respectively, at a local market in Worcester, MA, and from
ICN Biomedicals, Inc. (Aurora, OH). Crocetin was obtained from
Sigma Chemical Co. (St Louis, MO) and safranal was purchased from
Aldrich Chemical Company, Inc. (Milwaukee, WI). Other standards
were not available in the U.S.

Quantification. The saffron secondary products extracted from stig­
mas were quantified using a Hitachi U-2001 UV-visible, double-beam
spectrophotometer (Tokyo, Japan). The four key pigments, crocin,
picrocrocin, crocetin, and safranal, were identified from the absorption
maxima and quantified using respective extinction coefficients ob­tained from the literature (23) for crocin and picrocrocin and exper­
mentally from authentic standards for crocetin and safranal. Absorp­
tion maxima and extinction coefficients used were: crocin, 443 nm,
89,000 l mol⁻¹ cm⁻¹; picrocrocin, 250.5 nm, 10,100 l mol⁻¹ cm⁻¹;
crocetin, 424 nm, 30,328 l mol⁻¹ cm⁻¹; safranal, 311 nm, 9,280 l
mol⁻¹ cm⁻¹.

Statistical analysis. Differences among the culture systems were
tested using an analysis of variance (ANOVA, 95% confidence level).
In instances where the ANOVA indicated rejection of the null hypoth­
esis, the Tukey test was used as a posteriori test. Means and standard
deviations for all measured parameters were calculated.

RESULTS

Saffron plants grown aeroponically and hydroponically for six weeks
produced corms with significantly less increase in fresh weight as
compared with corms from soil-grown control plants (Table 1). In
contrast, the change in dry weight of the corms grown in aeroponics
and hydroponics, was significantly higher as compared with soil con­
trols. In the hydroponic system, the biomass decrease was nearly 80
percent. The number of roots on the plants was significantly greater in
aeroponics as compared with the soil and hydroponic culture systems.
As compared with control plants in soil culture, maximum root length
TABLE 1. The growth and development of saffron bulbs.

<table>
<thead>
<tr>
<th>Growth variables</th>
<th>Aeroponics</th>
<th>Hydroponics</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight increase (%)</td>
<td>32 ± 2(^a)</td>
<td>11 ± 1(^b)</td>
<td>54 ± 3(^c)</td>
</tr>
<tr>
<td>Dry weight increase (%)</td>
<td>20.59 ± 0.01(^a)</td>
<td>25.64 ± 0.01(^b)</td>
<td>18.62 ± 0.03(^c)</td>
</tr>
<tr>
<td><strong>Root development</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots (No.)</td>
<td>43.82 ± 7.24(^a)</td>
<td>19.08 ± 4.72(^b)</td>
<td>37.07 ± 4.90(^c)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>4.73 ± 0.42(^a)</td>
<td>1.92 ± 0.32(^b)</td>
<td>11.10 ± 1.55(^c)</td>
</tr>
<tr>
<td><strong>Leaf development</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoots (No.)</td>
<td>5.04 ± 0.51(^a)</td>
<td>5.22 ± 0.43(^a)</td>
<td>5.00 ± 0.56(^a)</td>
</tr>
<tr>
<td>Leaves (No.)</td>
<td>15.99 ± 2.76(^a)</td>
<td>15.87 ± 1.16(^a)</td>
<td>14.43 ± 3.43(^a)</td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>17.94 ± 4.78(^a)</td>
<td>17.70 ± 1.21(^a)</td>
<td>23.40 ± 8.11(^b)</td>
</tr>
<tr>
<td>Emergence rate (days)</td>
<td>19.88 ± 7.38(^a)</td>
<td>18.77 ± 6.09(^a)</td>
<td>24.16 ± 8.78(^b)</td>
</tr>
<tr>
<td><strong>Flowers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowering at anthesis (%)</td>
<td>8.3</td>
<td>4.17</td>
<td>12.12</td>
</tr>
<tr>
<td>Stigmata length (mm)</td>
<td>33 ± 4</td>
<td>35</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>Stigmata F.Wt. (mg)</td>
<td>22.20 ± 5.3(^a)</td>
<td>19.95 ± 9.90(^a)</td>
<td>21.52 ± 8.90(^b)</td>
</tr>
<tr>
<td>Stigmata D.Wt. (mg)</td>
<td>4.70 ± 0.53(^a)</td>
<td>4.95 ± 2.47(^a)</td>
<td>4.42 ± 0.85(^a)</td>
</tr>
</tbody>
</table>

1 As measured after six weeks growth; means ± SD; means within a row with the same letter are not significantly different from each other (P < 0.05).
2 Based on initial fresh weight of corms.
3 Based on initial dry weight of corms.
4 Days to 50 percent emergence.

in both soilless systems was significantly reduced having shorter, thicker, curlier, and a greater number of contractile roots than observed on plants in soil culture (data not shown). In the hydroponic system, roots grew directly in contact with the NFT channel. In the aeroponic system, root growth stopped when the roots reached 5 cm in length, corresponding to the depth of the growth chamber.

No significant differences in the number of shoots and leaves produced per corm was noted between the soil control and soilless cultures. The length of the leaves produced by the bulbs grown in soil, however, was significantly greater by 30 percent than the length of leaves on plants in the soilless cultures. Shoot appearance was delayed by four to five days on plants in soil cultures as compared with plants in aeroponic and hydroponic culture (data not shown).

The percentage of bulbs that developed flowers was very low in the nutrient cultures as compared with the soil cultures. Despite poor flowering, the fresh and dry weights of the stigmas harvested in the three culture systems were not significantly different.

A comparison of pigments in the stigmas harvested from aeroponic-
cally and hydroponically grown saffron indicated these tissues shared morphological and biochemical similarity to stigmas harvested from soil cultures and commercial saffron (Table 2). Among the tested culture systems, crocin and crocetin concentrations were greatest in stigmas produced from aeroponically grown saffron bulbs. Picrocrocin concentration was higher in stigmas from plants grown in soil culture as compared with stigmas from plants grown in nutrient culture. Safranal, present at low concentrations in each culture system, was likely volatilized during drying of the stigmata.

**DISCUSSION**

A number of factors influence the flowering of bulbous plants: temperature, relative humidity, storage time, bulb size, soil temperature, water stress, light intensity, and defoliation (25). For saffron plants, temperature, relative humidity during storage, and size and weight of the bulbs are particularly important for flowering (11,15). Leaving the corms in the ground between the first and second season also promotes more flowering, as does moist storage at 15°C, thus

<table>
<thead>
<tr>
<th>Secondary product</th>
<th>Aeroponics</th>
<th>Hydroponics</th>
<th>Soil</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh tissue</td>
<td>4.7 ± 0.4</td>
<td>4.0</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>Crocin</td>
<td>Dry tissue</td>
<td>22.1 ± 2.1</td>
<td>16.4</td>
<td>20.3 ± 5.1</td>
</tr>
<tr>
<td>Crocetin</td>
<td>Fresh tissue</td>
<td>4.3 ± 0.4</td>
<td>3.7</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Dry tissue</td>
<td>20.2 ± 1.7</td>
<td>15.2</td>
<td>16.0 ± 4.7</td>
</tr>
<tr>
<td>Picrocrocin</td>
<td>Fresh tissue</td>
<td>3.7 ± 0.6</td>
<td>3.8</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Dry tissue</td>
<td>17.4 ± 2.9</td>
<td>15.5</td>
<td>20.5 ± 8.2</td>
</tr>
<tr>
<td>Safranal</td>
<td>Fresh tissue</td>
<td>1.1 ± 0.1</td>
<td>1.2</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Dry tissue</td>
<td>5.3 ± 0.7</td>
<td>4.9</td>
<td>4.6 ± 2.2</td>
</tr>
</tbody>
</table>

^1 As measured after six weeks growth; all samples measured spectrophotically in an ethanol extract of harvested stigmas; means ± SD.
^2 Commercial sample purchased at local market in Worcester, MA.
^3 Only two flowers were harvested and only one extraction was measured.
^4 Sample not available.
raising the flowering capacity of the plant (12). After being received by us, the bulbs in our study were kept at room temperature before use but, unfortunately, their earlier history was unknown.

Saffron corms >2.5 cm diameter are reported to bloom at a level of 15 to 20 percent and small corms (< 6.9 g) do not flower within two seasons after planting (11,14). Indeed, we observed that only corms larger than 2.6 cm bloomed (data not shown). Mc Gimpsey et al. (11) observed that corms < 6.9 g did not flower in the two seasons after planting and confirmed that corm weight plays a critical role in saffron flowering.

The increase in fresh saffron corm biomass observed after six weeks growth in soil, as compared with soilless cultures, was due to a greater water content in the corms grown in soil culture. The higher fresh weight of corms in soil culture did not lead to a significantly greater dry weight in these corms. Potato plantlets grown aeroponically have shown a similar response to nutrient as opposed to soil culture (10).

A decrease in root length in plants growing in nutrient culture systems, a response noted in saffron plants, as compared with soil culture, has been previously observed in bean plants grown hydroponically and aeroponically (3). These decreases in root length in saffron plants grown in nutrient culture may be due to light reaching the corms in the hydroponic and aeroponic culture systems since the saffron corms were planted relatively shallow in these situations, covered by only 2 cm of Leca Stone. Light is known to have an inhibitory effect on root growth (16). A reduced root system could have negative effects on nutrient and water uptake leading to reduction in plant growth, probably accounting for the significant decrease in fresh biomass of corms observed in the nutrient culture systems. Inhibition of root development, a problem in hydroponic growth of plants, has been thought to be mainly due to an insufficient oxygen supply to the roots that leads to hypoxia and depressed root growth (6,21).

The unusual root morphology observed in aeroponic and hydroponic cultures is probably related to mechanical resistance impeding the growth of the roots in hydroponic culture (29). Mechanically impeded roots have been reported to be shorter, thicker, and more irregularly shaped than roots grown in soil (1). Such mechanical impedance would be associated with the NFT channel and the wall of the mist chamber in the hydroponic and aeroponic systems, respectively.
The formation of contractile roots in saffron bulbs grown aeroponically and hydroponically correlated with a previous report by Halevy (8) that indicated the induction of contractile roots in Gladiolus was inversely related to planting depth and promoted by light intensity (8). Many bulbs and cormous geophytes have underground movement, achieved by the formation of contractile roots (17), to reach a desired depth. In saffron plants, formation of contractile roots plays a function in corm lowering (12).

Negbi et al. (12) showed that the rate of emergence of saffron plant shoots was inversely related to planting depth. Our results also demonstrated that leaf elongation was directly related to planting depth. Shoot appearance in aeroponic and hydroponic systems, where planting depth was shallow as compared with planting depth in soil, was earlier than shoot emergence in soil culture. No significant differences in the number of shoots or leaves produced per bulb, however, was observed among all three culture systems.

Saffron corms growing in soil culture almost reached the expected level of flowering (12.1%) for plants growing in commercial fields. In contrast, corms grown aeroponically and hydroponically blossomed significantly less (8.3 and 4.2%, respectively). The reasons for the decrease in flowering are unknown, but suggest some significant effects due to environmental changes or differences between soilless conditions and soil culture. Stigma weights in plants growing in the nutrient and soil cultures were slightly lower than that of stigmas harvested from field plants (15). Raina et al. (18) observed that the average weight of fresh saffron stigmas varied from 26 to 37 mg and the length varied from 28 to 35 mm. Stigmas obtained from the aeroponic and hydroponic cultures and the soil culture were > 30 mm long with a hard, brilliant color, indicating a “very select” grade according to the grading system defined by Sampathu et al. (20).

Levels of crocin, crocetin, picrocrocin, and safranal in nutrient and soil cultured plants in our experiments were similar to those published by Sujata et al. (23). Compared with pigment levels observed using in vitro technology, our production in stigmas at anthesis was 500 to 1000 times higher (26). Rains et al. (8) noted a crocin content of between 0 and 0.68 mg/mg fresh weight, depending on the processing conditions after harvest. These concentrations are slightly higher than ours and most likely due to differences in our methods of analysis (18,22).
Provided that the corms used in propagation are large enough, our results suggest that nutrient culture can be used to produce high quality saffron. Further studies should be undertaken to maximize flowering, and, perhaps hysteranthy, to optimize saffron production in controlled environments.

REFERENCES


