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STUDIES ON SAFFRON IN KASHMIR. III. IN VITRO PRODUCTION OF CORM AND SHOOT LIKE STRUCTURES

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ABSTRACT

Callus in saffron was induced on Murashige and Skoog medium supplemented with 2 mg/l each of 2,4-D and kinetin. Proliferation of callus and differentiation into corm, shoot and flower-like structures was achieved on the same medium supplemented with 1 mg/l NAA and 2 mg/l kinetin.

Key words: *Crocus sativus* L., saffron, callus, in-vitro culture.

Saffron (*Crocus sativus* L.) cultivation in India is mainly confined to the State of Jammu and Kashmir and is spread over an area of about 5,000 ha. The cultivation of saffron in the state did not receive any scientific attention till 1980 [1]. Dhar et al. [2] reported triploid nature of the Kashmir cultivar (2n=3x=24). The existence of sterility barrier precludes any immediate improvement. Induction of callus and subsequent regeneration of plants is suggested as a possible means of introducing new variation. However, practically no information is available on the in vitro culture of saffron. In the present paper the in vitro response of different explants and its differentiation into corm, shoot and flower like structures is discussed.

MATERIALS AND METHODS

The sprouted corms of *Crocus sativus* L. growing at our farm were washed with water and cleared of its scales. Corms were cut horizontally into three or four slices each of which was further cut into segments with and without an axillary bud. The elongated corms were dissected to expose floral and vegetative primordia. Explants in addition to corm pieces consisted of leaf segments, floral and vegetative primordia, each about 1 cm long. These explants were surface sterilized with 0.1% mercuric chloride for 2 min and washed in sterile

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double distilled water. The sterilized explants were inoculated on Murashige and Skoog (MS) medium [3] containing 2% sucrose. The basal medium was supplemented with 1-3 mg/l naphthoxy acetic acid (NAA), 2-5 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D), and 1-5 mg/l kinetin. The pH of the medium was adjusted to 5.8 and solidified with 0.9% agar. The cultures were maintained at constant temperature of 22 ± 2°C. The usual power failure during both 1987 and 1988 could not be avoided. However, temperature in all cases never exceeded 30°C, but sometimes dropped to 8°C. The experiment was repeated thrice in 1987, 1988 and 1989, with six replicates each time. For recording the growth data, fifteen 93-100 mg pieces of fresh callus were cultured in Erlenmeyer flasks and fresh weight of callus was recorded for every three cultures after fortnightly intervals and later expressed on mean basis.

RESULTS AND DISCUSSION

Of the various explants used, only the floral apices responded to callus production. Callus was induced from the explants on MS medium supplemented with 2 mg/l each of 2,4-D and kinetin. The material increased in volume and in certain cases produced a flowering tube (perhaps due to the elongation of cells of the flowering primordia). Due to browning the material was transferred to the same medium supplemented with 100 mg/l ascorbic acid. There was only a slight increase in its growth and the material was transferred to MS medium supplemented with 2 mg/l kinetin and 1 mg/l NAA. The NAA-supplemented medium helped proliferation of callus (Fig. 1: 1).

The growth of callus was, however, slow. The average time required for doubling the mass of callus tissue was 8 weeks (Table 1). However, the initial size (measured by fresh weight) of the callus was important and callus tissues of less than 50 mg weight showed erratic response. All callus was nonchlorophyllous, brownish white, firm but turned yellowish brown as it became old. There was consistency in the results of experiments repeated each year. Further subculturing of the callus resulted in the formation of corm and shoot-like structures (Fig. 1: 2). Some of these shoot-like structures produced cup shaped structures at the top.

Table 1. Growth of uniformly cut cultures of saffron callus

No. of days of growth	Fresh wt. of callus, g	Standard deviation	Coefficient of variability
0	96.2	2.98	3.10
15	104.0	6.48	6.23
30	131.2	8.54	6.50
45	167.5	11.59	6.92
60	203.2	15.77	7.75

Histological observation revealed meristemoid formation during early differentiation in the parenchymatous mass. These meristemoids are organised into regular pattern in the

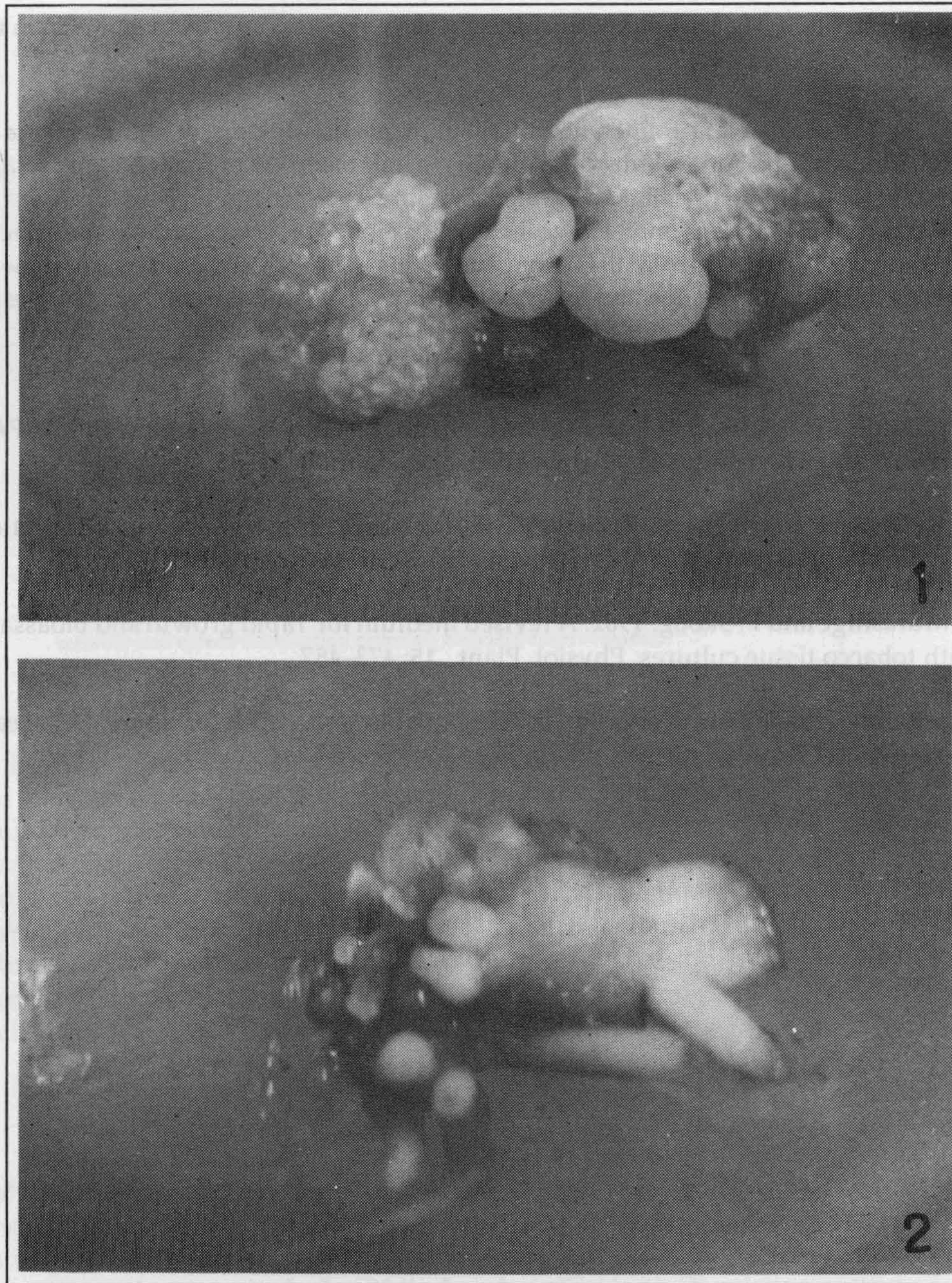

Fig. 1. **Callus development in saffron. 1) Profuse development of callus and corm-like structures. 2) Differentiation of callus into corm and shoot-like structures.**

shoot-like structures. The corm-like structures produced roots which were pentarch in the cross-section.

Hussey [4] studied the totipotency of tissue explants of some of the members of Liliaceae, Iridaceae and Amaryllidaceae. Among the four members of Iridaceae, *Schizostylis* took 65 days for doubling its callus while the remaining three genera, i.e. *Freessia, Gladiolus,* and *Sparazis* took 30–40 days only. However, *Freessia* alone was consistently responsive to regeneration and produced plantlets from the callus. The present study also showed consistency in callus production and formation of shoot-like structures in *Crocus sativus*.

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