

IN VITRO REGENERATION OF SHOOT AND CORM FROM THE DIFFERENT EXPLANTS OF *CROCUS SATIVUS* L.

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Abstract

Different explants of *Crocus sativus* L. such as corm, style, ovary, petal, anther and leaf were cultured on LS and MS media supplemented with NAA and BAP or 2,4-D plus BAP at different combinations. Shoot and corm induction occurred in addition to the formation of other organs. Efficient plantlet regeneration with strong roots occurred at 1 mg l⁻¹ of both NAA and BAP. An increase in 2,4-D level enhanced callus formation, but suppressed shoot bud generation. Shoot and corm regeneration was observed on other explants in addition to corms.

Introduction

Crocus sativus L. (Iridaceae) is cultivated for its red three-lobate stigmata containing saffron. This plant blooms in the autumn and the daughter corm initiation and development take place during November-April. The natural reproduction rate of new corms by conventional culture practice is quite low. The propagation of corms is necessary because of the triploid nature of *C. sativus* [1].

The saffron *Crocus* is known to be infested with pathogenic fungi and viruses which are carried over by the corms and may affect the crop under certain conditions [2]. Any attempt to improve and modernize the cultivation of saffron will, therefore, require efficient mass production of pathogen-free corms, for which purpose tissue culture is ideal. The *in vitro* propagation of pathogen-free organs has been successful in some geophytes of Iridaceae, Amarylidaceae and Liliaceae [2].

Tissue culture techniques have recently been used on *Crocus*, but with limited success. Efforts to produce corm and to induce plantlet regeneration, *in vitro*, were carried out recently, however there is no report about root induction [4,5,6]. This paper deals with shoot and corm forma-

tion on different explants and the regeneration of the whole plantlet with strong roots on corm explant of *C. sativus* for the first time.

Materials and Methods

Flowering corms of *Crocus sativus* L. were collected from the saffron field and brought into the laboratory in November 1993. The corms were descaled and washed in running tap water, and the flower buds were excised and sterilized in 70% ethanol for 5 min.

The buds were then treated with sodium hypochlorite (containing chlorine 2.5%) for 15 min and rinsed three times with sterile distilled water. After being dissected aseptically from the shoots, the flower buds were separated into styles, ovaries, petals, anthers and leaves. The explants were planted on Linsmaier and Skoog medium [7], with 0.9% agar, 3% sucrose and NAA and BAP at concentrations of 0.1, 1.5, 10 and 0.1, 0.2, 1.5, 10 mg l⁻¹, respectively.

Corms with diameters less than 1.5 cm were collected. After being washed in running tap water, they were surface sterilized in 70% ethanol for 15 min and in sodium hypochlorite (containing chlorine 2.5%) for 30 min, then rinsed three times in sterile distilled water. Corm frag-

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ments were aseptically cultured on Murashige and Skoog medium [8], with 0.9% agar, containing 3% sucrose and NAA and BAP at concentrations of 0.1, 1.5, 10 mg l⁻¹ and/or 2,4-D and BAP at concentrations of 0.25, 0.5, 1, 2 mg l⁻¹. The pH of all media was adjusted to 5.7-5.8 prior to autoclaving. All cultures were maintained in the dark at 25±°C. After 30 days, the explants were subcultured on the same medium. After formation of leaf organs on corm explants, cultures were transferred to the light as needed.

Results and Discussion

In all LS media, style explants of *C. sativus* produced neoformed corms in N1-B1 and N1-B5 media. In LS (N1-B1) medium, style explants formed callus and contractile roots after the first subculture. After six months, neoformed buds appeared and after a year, new corms were formed at the base of the buds (Fig. 1,A). The response of style explant in LS (N1-B5) medium was better than in LS (N1-B1) medium. After 75 days, leaf-like organs with new

corms at their base were observed on style explants in this medium (Fig. 1,B).

Shoot regeneration occurred on style explants in LS (N5-B1) medium in addition to former media. The callus induced on LS medium containing 5 mg l⁻¹ NAA and 1 mg l⁻¹ BAP produced shoots, and after eight months shoots exhibited further growth.

In LS (N5-B1), besides shoot and corm formation, stigma, corm-like structures and contractile roots were also produced. In other hormonal combinations, shoot and corm regeneration did not occur. Ovary explants produced shoots in LS (N1-B1), LS (N10-B5) and LS (N5-B1) media. In LS (N1-B1) medium, after formation of callus and contractile roots, shoots were regenerated after eight months (Fig. 2, A). Shoot regeneration from the callus formed on ovary explants in LS (N10-B5) medium occurred after a year (Fig. 2, B). At the same time, on the half-ovary explants with cross cutting, shoot formation was observed in LS(N5-B1) medium (Fig. 2,C).



Figure 1. Production of neoformed organs on callus-tissue formed on style explants

A= New corm at the base of neoformed bud in LS (N1-B1) medium after one year. B= Leaf-like organs and neoformed buds in LS (N1-B5) medium after 75 days

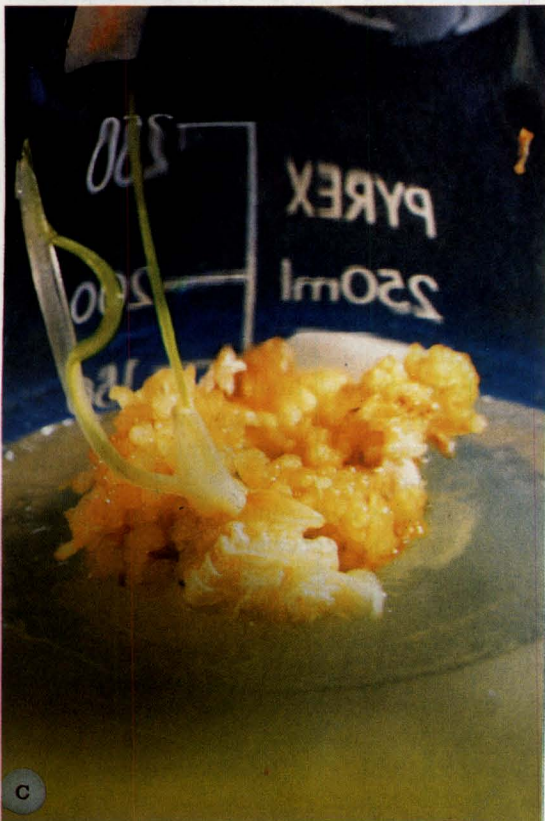
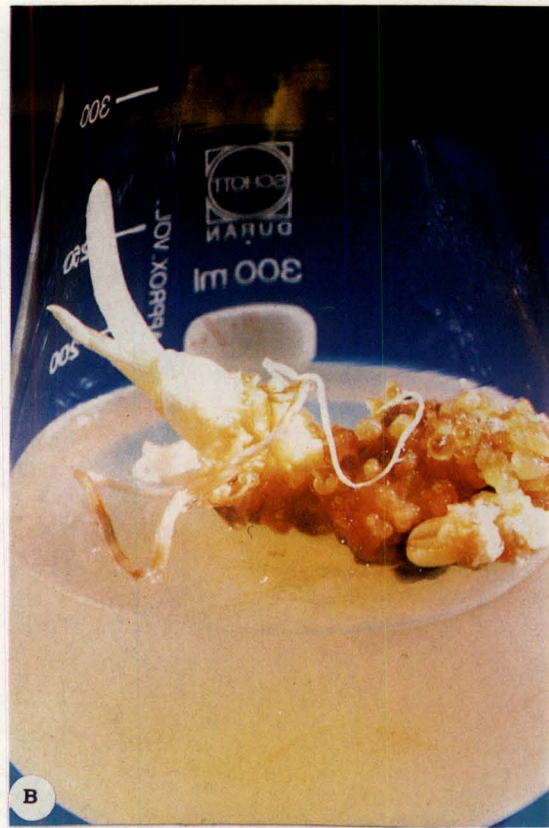


Figure 2. Production of neoformed organs on callus tissue formed on ovary explants

A= Shoot regeneration in LS (N1-B1) medium after eight months (contractile roots are also evident). B = Nodulated callus with neoformed bud on callus in LS (N10-B5) medium after one year. C= Nodulated callus with green, leaf-like organs with cross cutting in LS (N5-B1) medium after a year

New corms formed at the base of neoformed buds only in LS (N1-B1) medium. Shoot and corm regeneration was evident on the petal explant of *C. sativus* only in LS(N10-B5) medium. In this medium, neoformed buds were produced on callus formed on petal explant after eight months. After 15 months, callus exhibited further growth and shoots were greater (Fig. 3, A). After 17 months, neoformed corms were observed at the base of shoots (Fig. 3, B).

Anther and leaf explants did not produce any shoots and/or corms. Shoot regeneration was observed on corm explants of *C. sativus* in several hormonal combinations. Corm explants produced shoots when 2,4-D at 0.25, 0.5 and 1 mg^l⁻¹ and BAP at 0.25 and 2 mg^l⁻¹ were used (Fig. 4, A, B).

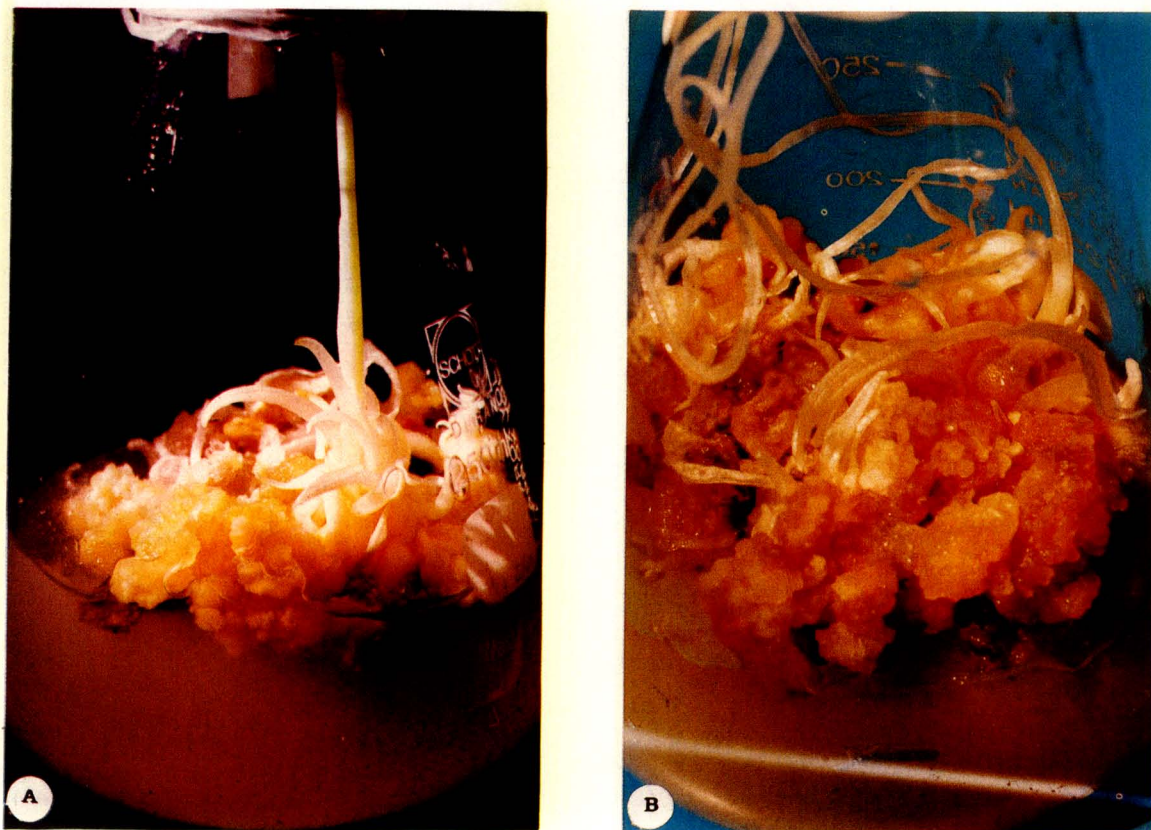


Figure 3. Production of neoformed organs on callus tissue on petal explants

A = Copious callus and regeneration of shoot in LS (N10-B5) medium after 15 months. B = Extension of callus and leaf-like organs in LS (N10-B5) medium after 17 months. Neoformed buds are also shown.

The best overall results from all the media used were obtained from MS (D1-B0.5) medium. When NAA and BAP were used, shoot regeneration was observed in MS (N1-B1), MS (N5-B1), MS (N5-B5), MS (N0.1-B1) and MS(N0.1-B5) media. In MS (N1-B1) medium, after shoot regeneration from callus, contractile roots appeared after eight months (Fig. 4, C). After nine months, some green, leaf-like organs were observed. After 10 months, complete neoformed corms formed (Fig. 4, D). These corms were completely similar to natural corm after 19 months.

Using MS (N5-B1) medium, shoot buds were formed on callus produced from corm explants, after four months (Fig. 4, E). After 10 months, further leaf-like organs were observed (Fig. 4, F). Neoformation of buds also occurred in MS (N5-B5) medium (Fig. 4, G). In MS (N0.1-B1) medium, shoot regeneration occurred on callus produced on

corm explants. Shoot regeneration was also observed in MS (N0.1-B5) (Fig. 4, H) medium.

Results obtained from this experiment indicate that MS (N1-B1) is the best medium for plantlet regeneration. Corm formation occurred on corm explants better than on other explants (Table 1).

When corms were cultured, NAA and BAP were used at higher and equal concentrations (5 and 10 mg l⁻¹), which caused lower growth rate of shoot and leaf organs. BAP at 1 mg l⁻¹ was suitable to induce shoot formation and further growth, but corm induction occurred well at 1 mg l⁻¹ NAA. When 2,4-D and BAP were used, shoot regeneration was decreased by increasing 2,4-D concentration. When the concentration of BAP was increased, shoot induction was enhanced accordingly. The other explants were shown to be effective for induction of neoformed corm in addition to corm cultures.

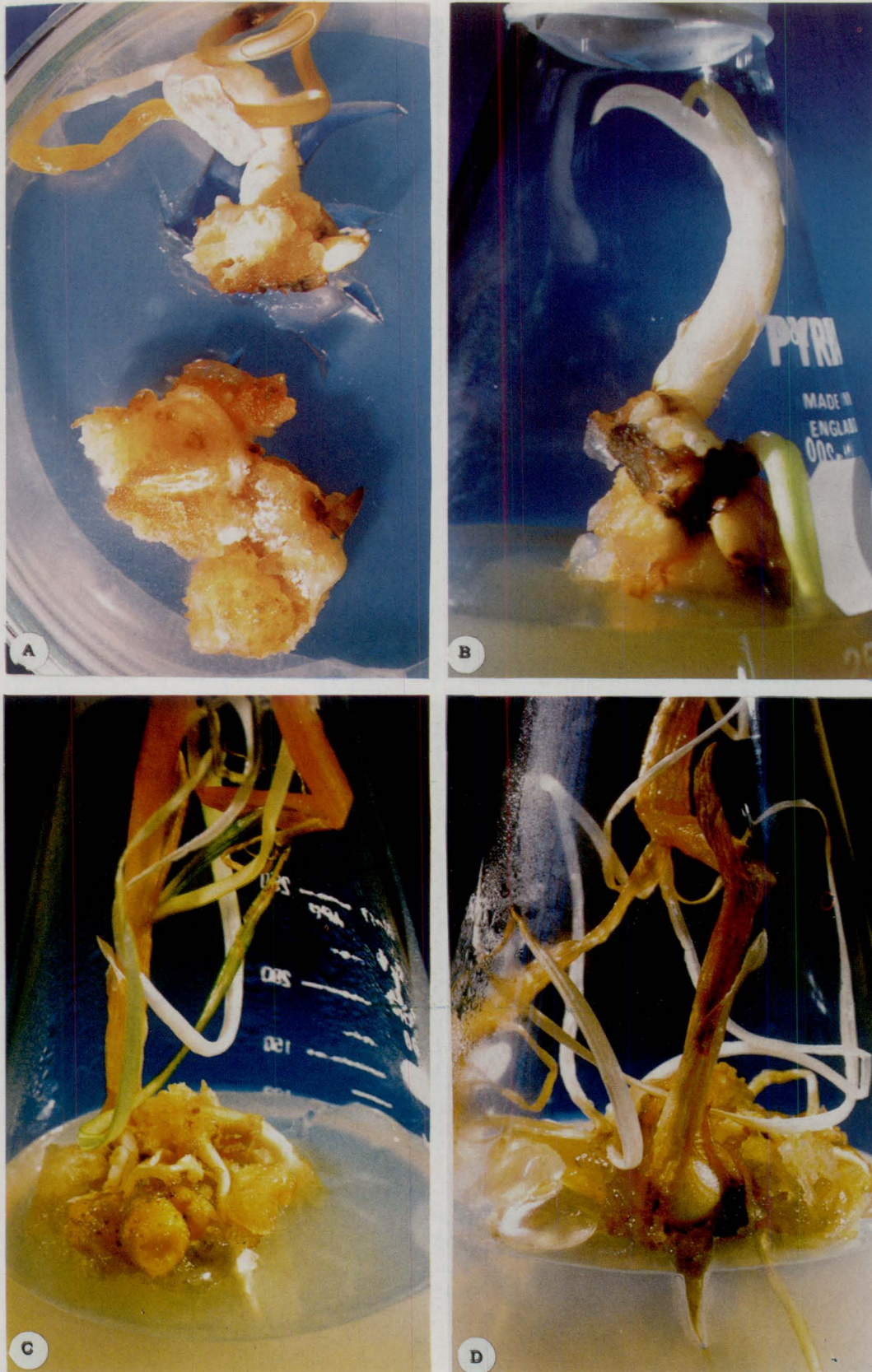


Figure 4

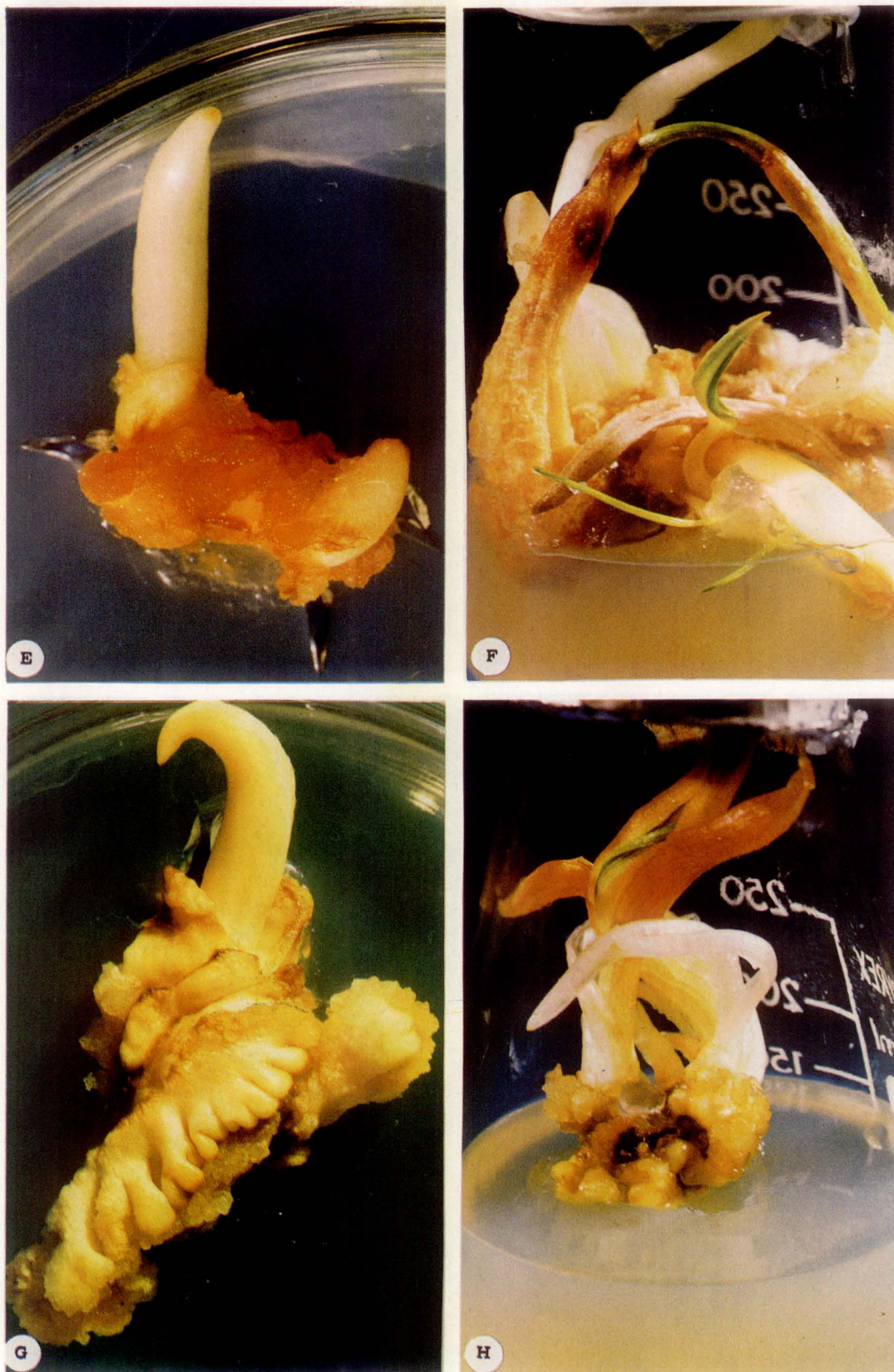


Figure 4 (Continued)

Figure 4. Production of neoformed organs on callus tissue formed on corm explants

A = Shoot and corm regeneration in MS (D0.25-B0.5) medium after one year. B = Shoot regeneration in MS (D0.25-B2) medium after eight months. C = Appearance of contractile roots and green, leaf-like organs in MS (N1-B1) medium after eight months. D = Neof ormation of corm in MS (N1-B1) medium after 10 months. E = Regeneration of shoot in MS (N5-B1) medium after four months. F = Extensive growth of leaf-like organs in MS (N5-B1) medium after ten months. G = Shoot regeneration on hard callus formed on corm explant in MS (N5-B5) medium after eight months. H = Regenerated shoot with contractile roots on hard callus in MS (N0.1-B1) medium after a year

Table 1. The comparison of morphogenetic potential of different explants of *C. sativus* in LS and MS media at various concentrations of NAA (N), 2,4-D(D) and BAP(B)

Explant	Medium	NAA(mg/l)	BAP (mg/l)	2,4-D (mg/l)	Shoot regeneration	Corm regeneration
Style	LS (10)	1	1	-	+	+
		1	5	-	+	++
		5	1	-	+	-
Ovary	LS	1	1	-	+	+
		5	1	-	+	-
		10	5	-	+	-
Petal	LS	10	5	-	+	+
Corm	MS (11)	-	0.5	0.25	+	+
		-	2	0.25	+	+
		-	0.25	0.5	+	+
		-	0.5	1	+	-
		1	1	-	+++	+++
		5	1	-	++	-
		5	5	-	+	-
		0.1	1	-	+	-
0.1	5	-	+	-		

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