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REGENERATION OF NEOFORMANT ORGANS FROM ORGAN-PARTS OF SOME *CROCUS* SPECIES

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Abstract

Explants of leaves, petals, anthers, styles and ovaries of *C. speciosus*, *C. cancellatus*, *C. gilanicus* and *C. almehensis* were cultured on LS media with three different combinations of NAA and BAP in the dark. Callus and neoformant organs (leaf and corm-like structure and ordinary and contractile roots) were formed on some of these explants. Corm explants from these species were also cultured on MS media with different combinations of NAA, BAP, Kin, zeatin and 2,4-D in the dark. Callus formation and corm, shoot and root regeneration were observed on corm explants from some of these species.

Introduction

Members of the genus *Crocus* are important in the horticultural industry as autumn, winter and spring flowering corms, whilst *Crocus sativus* is the source of saffron. *Crocus* is a genus of the Iridaceae family and several reports on *in vitro* culture have appeared involving members of this family. A substantial amount of work has been reported on *Gladiolus* and *Iris* [3], however, members of the genus *Crocus* have received little attention until recently and most of the reports centre on work done on *Crocus sativus*.

Fakhrai and Evans [2] were first to report on the *in vitro* culture of *Crocus chrysanthus*. They observed that when ovary explants were cultured on MS basal medium with 5.0 and 10 mg l^{-1} of BAP, they produced callus on the ovary surface and subsequently, stigma-like structures formed on the surface of this callus.

Upon transference to light, yellow pigmentation of the stigma-like structures resulted, whereupon they came to resemble the naturally-grown stigmata.

In this paper, we report on the *in vitro* morphogenetic potential of the various floral (petals, anthers, ovary, style) and corm tissues of four wild species of *Crocus*: *C. almehensis*, *C. speciosus*, *C. cancellatus* and *C. gilanicus*, for the production of neoformant organs.

Materials and Methods

Corms and flower buds of *C. speciosus*, *C. cancellatus*, *C. almehensis* and *C. gilanicus* were collected in Iran during the flowering season and brought into the laboratory. The flower buds were excised from corms and sterilized by dipping them in 70% ethanol for 5 min. They were then treated with sodium hypochlorite (containing chlorine 2.5%) for 15 min and rinsed three times with sterile water. The flower organs were aseptically dissected from the buds. Ovaries, styles, petals, anthers and leaves were carefully separated from each other. The explants

Keywords: *In vitro* culture; Organogenesis; Phytohormone; Plant propagation; Tissue culture

Abbreviations: Nand NAA= Naphthaleneacetic acid; Band BAP= Benzylaminopurine; K and Kin= Kinetin; D and 2,4-D= 2,4-Dichlorophenoxyacetic acid; Z= Zeatin

were planted on Linsmaier and Skoog [4] medium, with 0.9% agar, containing 3% sucrose and NAA and BAP in different combinations. The pH of the medium was adjusted to 5.7-5.8 prior to autoclaving. All cultures were maintained in the dark at 25±2°C. After 30 days the explants were subcultured on the same medium.

The corms of these species were rinsed in running tap water for 10 min then dipped in 70% ethanol for 15 min followed by treatment with sodium hypochlorite (containing chlorine 2.5%) for 30 min, and finally rinsed three times in sterile water. Corm fragments were aseptically cultured on Murashige and Skoog [6] medium, with 0.9% agar, containing 3% sucrose and various growth hormones (NAA, BAP, 2,4-D, zeatin, kinetin). The pH of the medium was adjusted to 5.7-5.8 prior to autoclaving. The cultures were maintained in the dark at 25±2°C. After 30 days the explants were subcultured on the same medium.

Results

The floral explants of *C. speciosus*, *C. almehensis* and *C. cancellatus* were cultured on LS media supplemented with NAA at a concentration of 5 mg l^{-1} and BAP at concentrations of 0.2, 1 and 5 mg l^{-1}. The growth rate of calli was derived from style explants of these three species and was lower than that of *C. sativus* (Table 1) as reported previously [1].

Among these species, *C. speciosus* showed a better response to each of the three hormonal combinations. After eight months in LS (N5-BO.2) medium, only hard calli were observed on style explants of this species (Fig. 1, A). After a year, the calli formed corm-like structures and contractile roots in this medium (Fig. 1, B). After eight months in LS (N5-Bl) medium, calli with leaf-like organs (white tubular structures) and contractile roots were observed on style explants of this species (Fig. 1, C). After a year, calli were greater, and many contractile and ordinary roots as well as a few corm-like structures were observed (Fig. 1, D). In LS (N5-B5) medium, only copious calli were observed on style explants of *C. speciosus* after a year (Fig. 1, E).

Style explants of *C. cancellatus* and *C. almehensis* responded similarly but only in LS(N5-B5) medium. After eight months, calli with few contractile roots were observed on style explants of *C. cancellatus* and after a year contractile and ordinary roots increased in this medium (Fig. 1, F,G). After eight months, only calli were observed

Table 1. The comparison of callus and neoformant organs formation on corm explants of wild species of *Crocus*

Species	Hormonal combination	Callus	Neoformant bud	Leaf-like organ	Contractile root	Corm-like structure
C. speciosus	N5-B0.2	+	-	-	+	+
	N5-B1	++	-	+	++	++
	N5-B5	+++	-	-	-	-
C. cancellatus	N5-B0.2	-	-	-	-	-
	N5-B1	-	-	-	-	-
	N5-B5	+	-	-	+++	-
C. almehensis	N5-B0.2	-	-	-	-	-
	N5-B1	-	-	-	-	-
	N5-B5	+	-	-	-	-

Figure 1. Callus and neoformant organs formation on style explants. A= Callus formation on style explant of *C. speciosus* in LS(N5-B0.2) medium after eight months; B= Corm-like structures and contractile roots on style explant of *C. speciosus* in LS(N5-B0.2) medium after a year; C= Callus with leaf-like organs and contractile roots on style explant of *C. speciosus* in LS(N5-B1) medium after eight months; D= Callus with many contractile and ordinary roots and a few corm-like structures on style explant of *C. speciosus* in LS(N5-B1) medium after a year; E= Callus without organ on style explant of *C. speciosus* in LS(N5-B5) medium after a year; F= Callus and contractile root formation on style explant of *C. cancellatus* in LS(N5-B5) medium after eight months; G= Many contractile and ordinary roots on style explant of *C. cancellatus* in LS(N5-B5) medium after a year; H= Callus without organ on style explant of *C. almehensis* in LS(N5-B5) medium after eight months

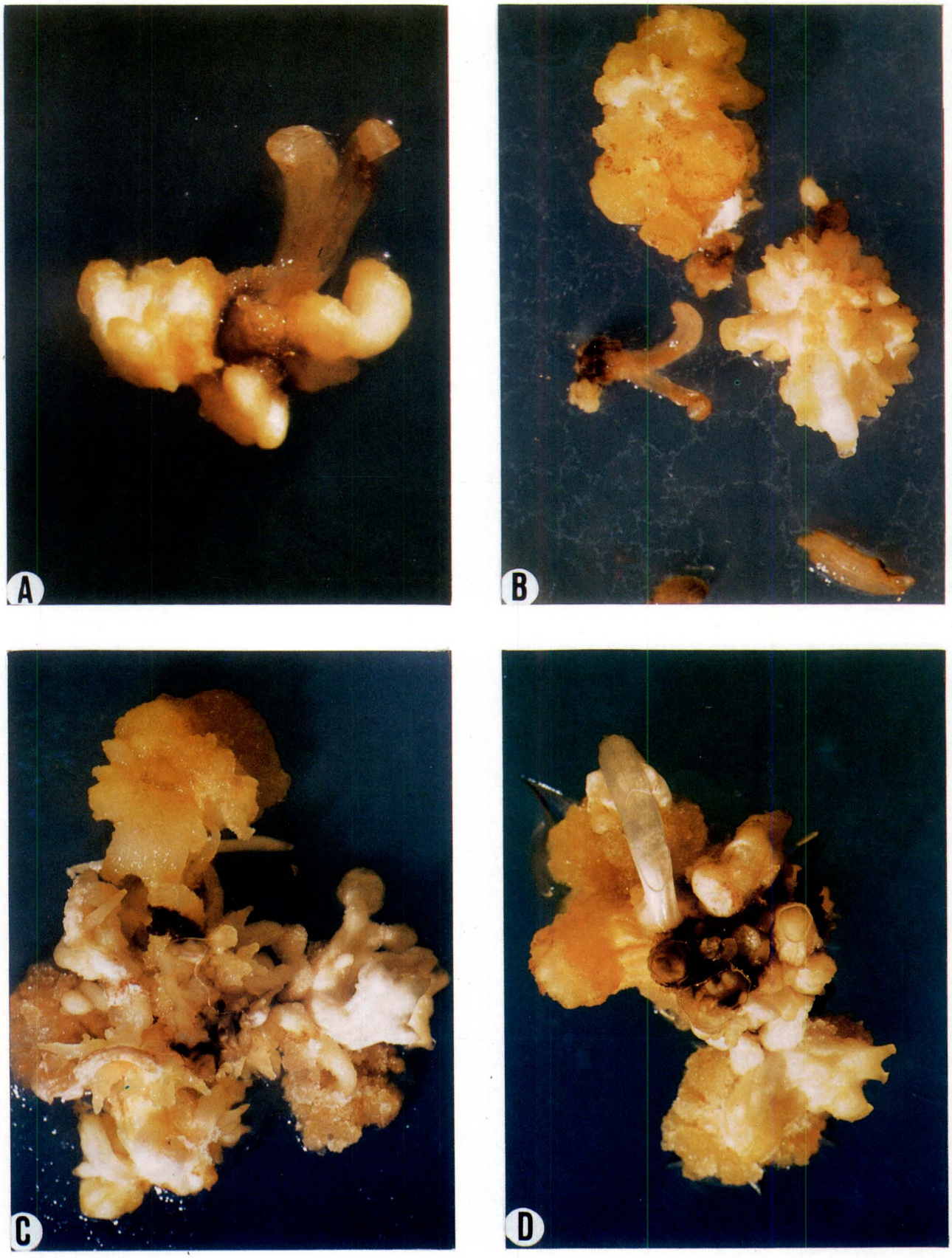


Figure 1

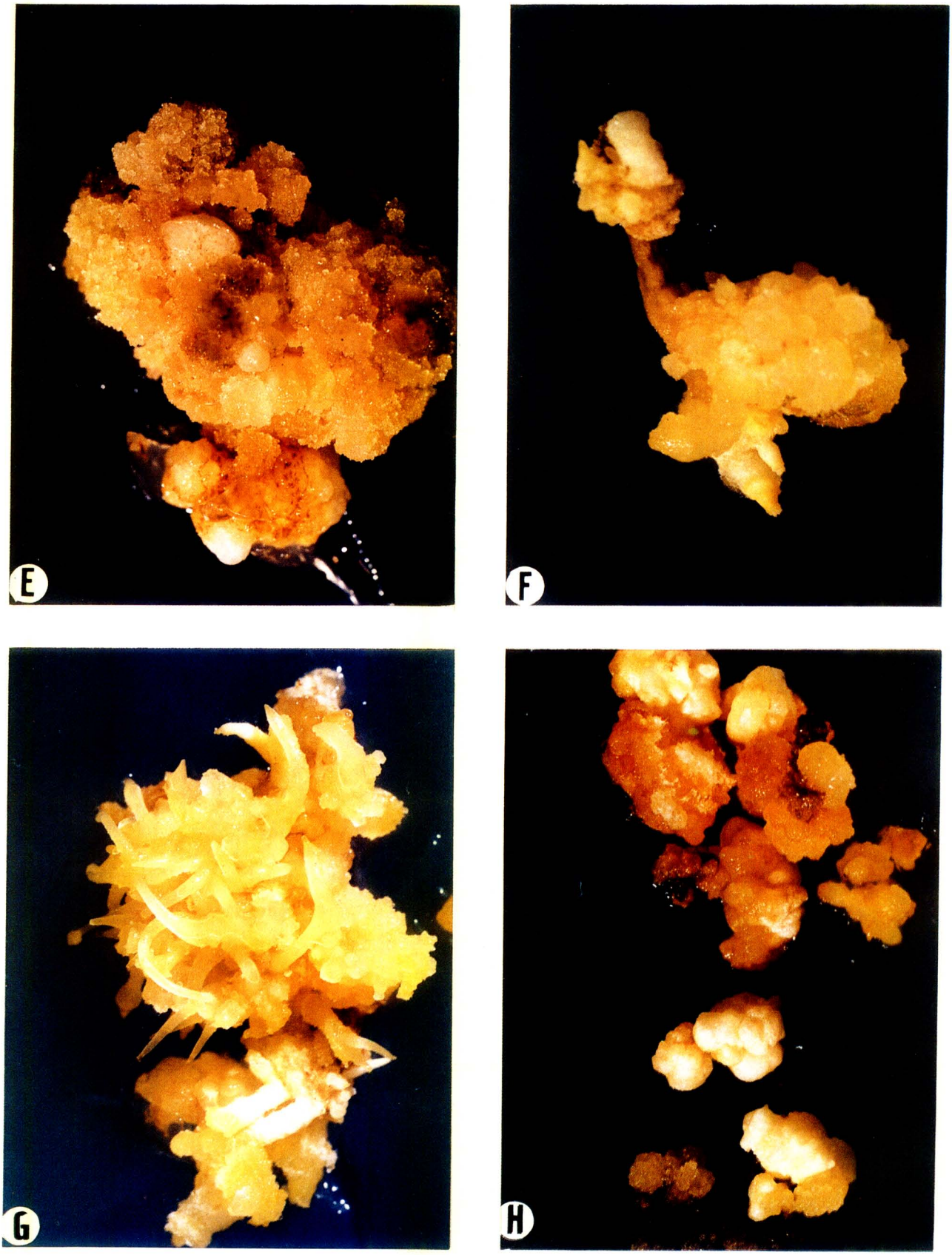


Figure 1 (Continued)

on style explants of *C. almehensis* and no organogenesis was observed in this medium (Fig. 1, H).

Among these species, no response was observed from ovary explants of *C. almehensis* (Table 2). Ovary explants of *C. speciosus* produced callus after one month in LS(N5-B0.2) medium. After the first subculture, these calli became greater and formed leaf-like organs (Fig. 2, A). After eight months, longer leaf-like organs and after a year, contractile and ordinary roots were observed on the calli (Fig. 2, B, C).

In LS(N5-Bl) and LS(N5-B5) media, ovary explants of *C. speciosus* produced slight callus after three months. In LS(N10-Bl) medium, ovary explants of *C. cancellatus* produced callus after six months. After eight months, callus became hard and nodular (Fig. 2, D).

Among these wild species of *Crocus*, only petal explants of *C. almehensis* responded to LS(N5-B5) medium. After eight months, only callus was observed on petal explants in this medium (Fig. 3).

In anther culture, only *C. almehensis* responded to LS(N5-Bl) medium. Anthers of this species formed only callus after two months (Fig. 4, A). After five months, calli became greater and no organogenesis was observed on them (Fig. 4, B).

Among these wild species, only young leaf explants of *C. gilanicus* showed a response to LS(N5-B0.2) medium. After one month, leaf explants formed callus (Fig. 5).

Corm explants of four wild species of *Crocus* were cultured on MS medium supplemented with two hormones from NAA, BAP, Kin, 2,4-D, zeatin in different combinations (Table 3). After 10 months, only slight callus formation was observed on corm explants of *C. speciosus* in MS (D0.5-Z0.3) medium (Fig. 6,A). These explants started swelling four weeks after culture in MS(N1-B1) medium. After two months, slight callus formation and after six months short leaf-like organs were observed on these explants in MS(N1-B1) medium. After nine months, neoformant bud with swollen base and roots was observed (Fig. 6, B). In MS (NS-B1) these explants formed callus and neoformant bud after two and nine months, respectively (Fig. 6, C). In MS(N10-B1) medium, corm explants of this species formed callus after two months. After nine months, great calli with neoformant bud were observed. The base of this bud swelled and became hard and formed a complete corm after a year (Fig. 6, D).

In *C. cancellatus*, corm explants showed only slight callus formation after 10 months in MS(KO.1-D1) medium (Fig. 6, E). In MS(D0.5-Z0.3) medium, callus and a

Table 2. The comparison of callus and neoformant organs formation on style explants of wild species of *Crocus*

Species	Hormonal combination	Callus	Neoformant bud	Leaf-like organ	Contractile root	Corm-like structure
C. speciosus	N5-B0.2	+++	-	++	+	-
	N5-B1	-	-	-	-	-
	N5-B5	-	-	-	-	-
C. cancellatus	N5-B0.2	-	-	-	-	-
	N5-B1	-	-	-	-	-
	N5-B5	-	-	-	-	-
	N10-B1	++	-	-	-	-
C. almehensis	N5-B0.2	-	-	-	-	-
	N5-B1	-	-	-	-	-
	N5-B5	-	-	-	-	-

Figure 2. Callus and neoformant organs formation on ovary explants. A= Callus with short, leaf-like organs on ovary explant (with cross-section) of *C. speciosus* in LS(N5-B0.2) medium after first subculture; B= Callus with long, leaf-like organs on ovary explant (with cross-section) of *C. speciosus* in LS(N5-B0.2) medium after eight months; C= Callus with long, leaf-like organs and contractile and ordinary roots on ovary explant (with cross-section) of *C. speciosus* in LS(N5-B0.2) medium after a year; D= Nodular callus without organ on ovary explant of *C. cancellatus* in LS(N10-B1) medium after eight months

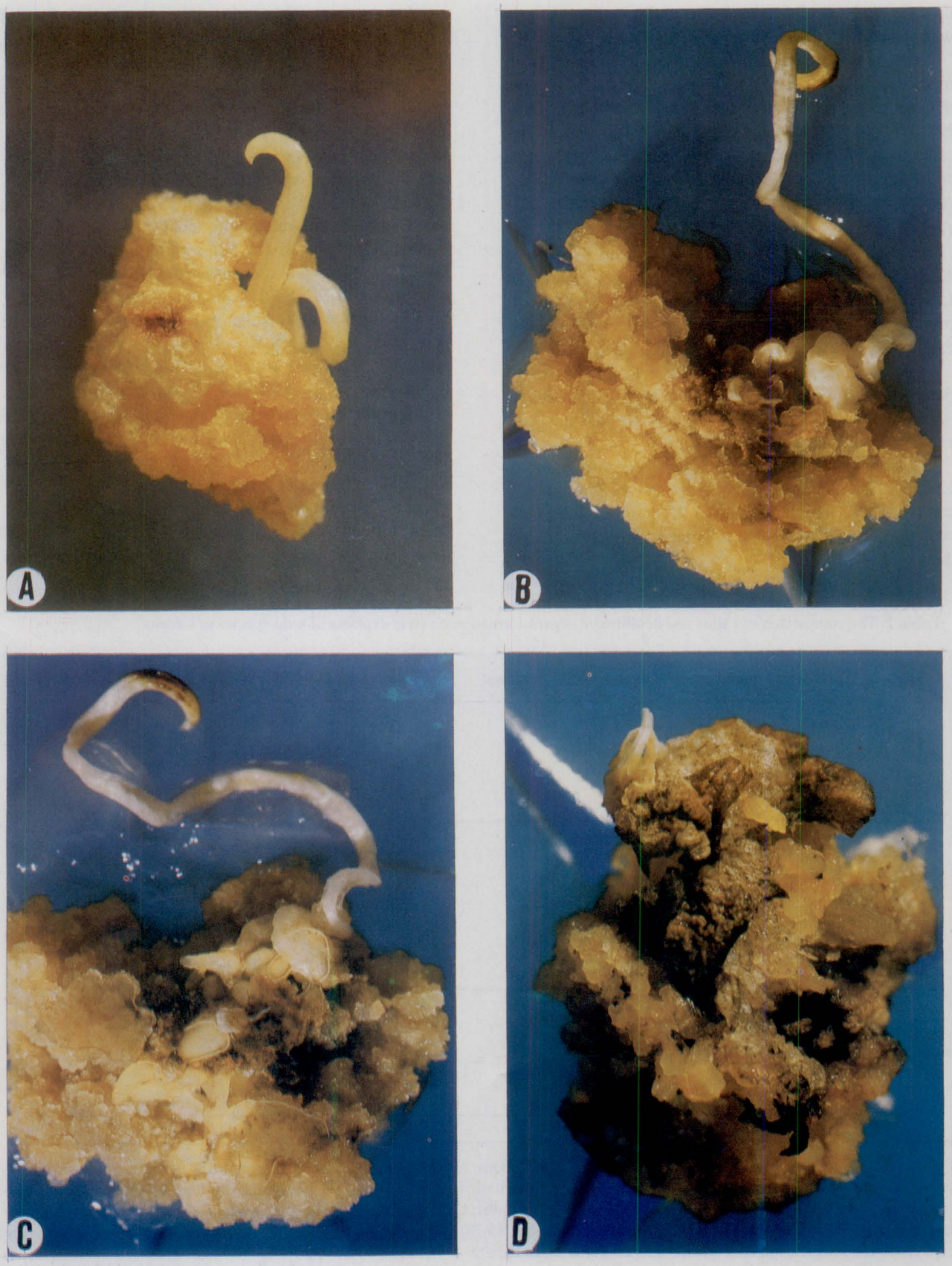


Figure 2

Figure 3. Callus on petal explant of *C. almehensis* in L(N5-B5) medium after eight months

few contractile roots and corm-like structures were observed after 10 months (Fig. 6, F). In MS(N1-B1) medium, these explants formed callus after one month. In MS(N5-B1) medium, these explants formed more callus than that formed in the latter medium after one month. After nine months, callus with leaf-like organs and many contractile and ordinary roots were observed (Fig. 6, G). After a year, contractile roots increased greatly (Fig. 6, H). In MS(N10-B1) medium, slight callus formation was observed on corm explants after one month and after nine months no organ was regenerated from calli (Fig. 6, I).

In *C. almehensis*, corm explants showed more callus formation in MS(N5-B1) medium than that in MS(N1-B1) medium after nine months (Fig. 6, J). In MS(N10-B1) medium, these explants only swelled after one month and after 10 months calli with very short leaf-like organs were observed (Fig. 6,K). Corm explants of *C. gilanicus* formed only callus in MS(K0.1-D1) after 10 months (Fig. 6, L).

Discussion

Among the four wild species of *Crocus* that were studied, style explants of *C. cancellatus* proved to be more potent for root formation and *C. almehensis* for callus formation (Table 1). Corm-like structures formed only on the calli which were derived from style explants of *C. speciosus*.

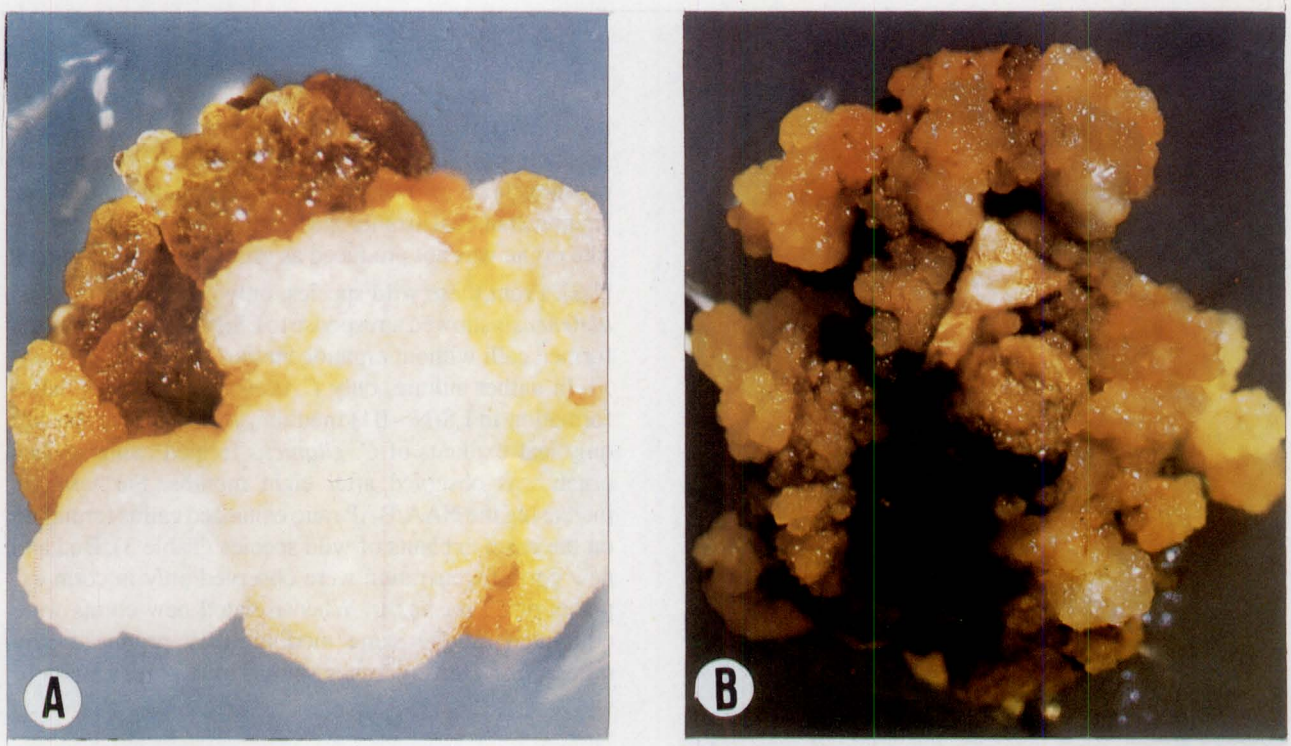
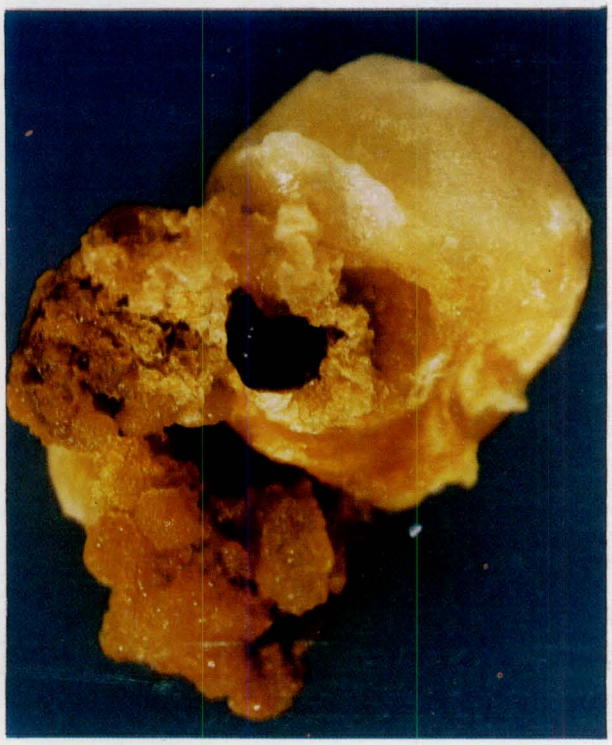


Figure 4. Callus formation on anther explant. A= *C. almehensis* in LS(N5-B1) medium after two months (x20); B= Callus without organ on anther explant of *C. almehensis* in LS(N5-B1) medium after five months

Table 3. The comparison of callus and neoformant organs formation on corm explants of wild species of *Crocus*

Species	Hormonal combination	Callus	Neoformant bud	Leaf-like organ	Contractile root	Corm-like structure	Neoformant corm
C. speciosus	D0.5-Z0.3	+	-	-	-	-	-
	D1-K0.1	-	-	-	-	-	-
	N1-B1	+	+	+	+	-	-
	N5-B1	++	+	++	-	-	-
	N10-B1	+++	+	+	-	-	+
C. cancellatus	D0.5-Z0.3	++	-	+	+	+	-
	D1-K0.1	+	-	+	-	-	-
	N1-B1	+	-	-	-	-	-
	N5-B1	++	-	+	+++	-	-
	N10-B1	+	-	-	-	-	-
C. almehensis	N1-B1	+	-	-	-	-	-
	N5-B1	++	-	-	-	-	-
	N10-B1	+++	-	+	-	-	-
C. gilanicus	K0.1-D1	+	-	-	-	-	-



The morphogenetic potential of style explants of *C. almehensis* is lower than that of other species for the production of neoformant organs. Callus formation and organogenesis on ovary explants of *C. speciosus* proved to be more than that in other species (Table 2), but the stigma-like organ was not produced as observed in other species [1,2]. From these wild species, only petal explants of *C. almehensis* showed a response to LS(N5-B5) medium and formed calli without organogenesis.

In anther culture, only *C. almehensis* showed callus formation in LS(N5-B1) medium. Among these species, only leaf explants of *C. gilanicus* formed callus and no organ was observed after eight months. Furthermore, increasing the NAA/BAP ratio enhanced callus formation on the corm explants of wild species (Table 3). Bud and new corm regeneration were observed only in corm explants of *C. speciosus*. When isolated new corms were cultivated on the same medium they produced new minicorms. This process is similar to the propagation of

Figure 5. Callus on leaf explant of *C. gilanicus* in LS(N5-0.2) medium after eight months

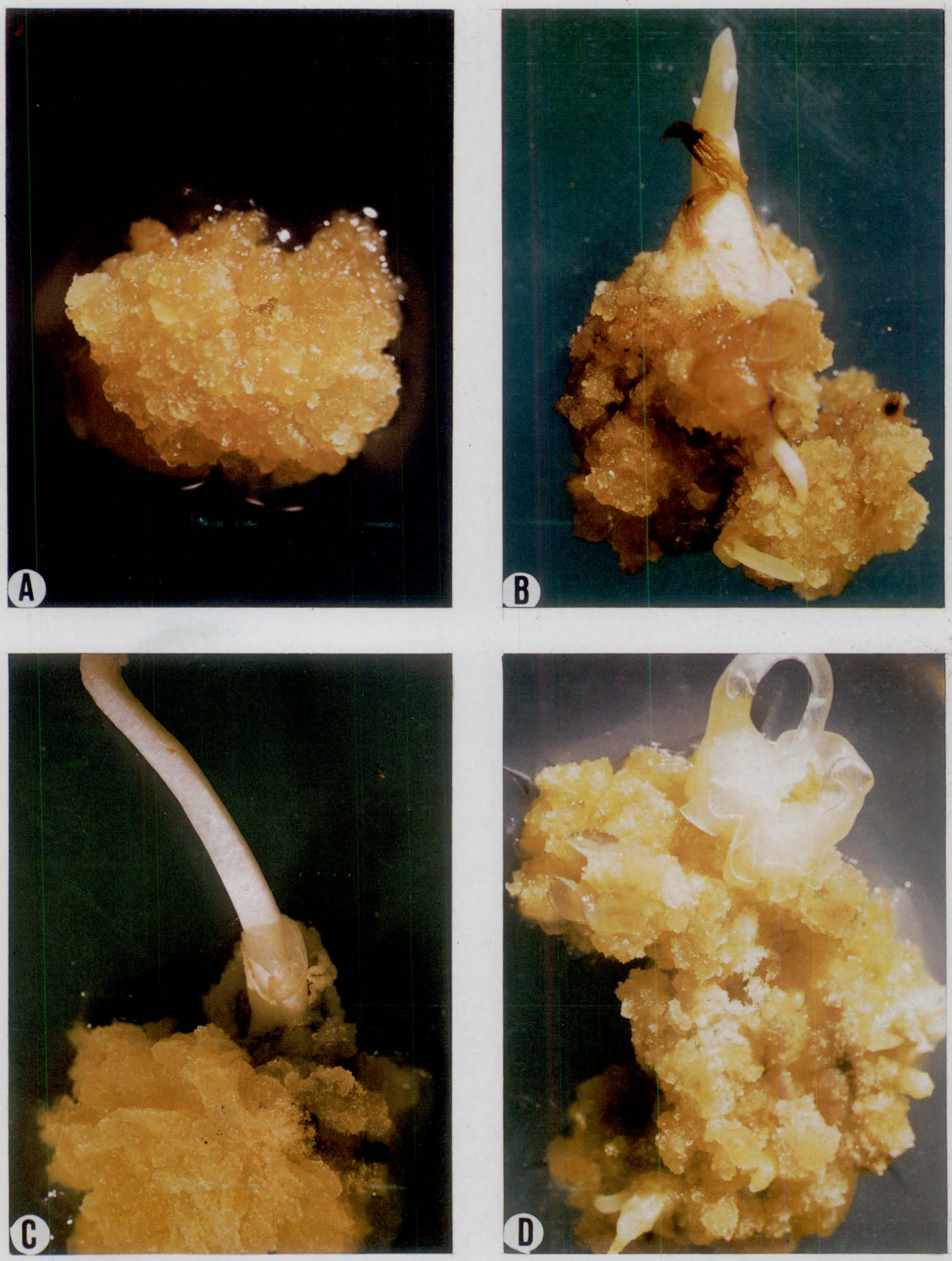
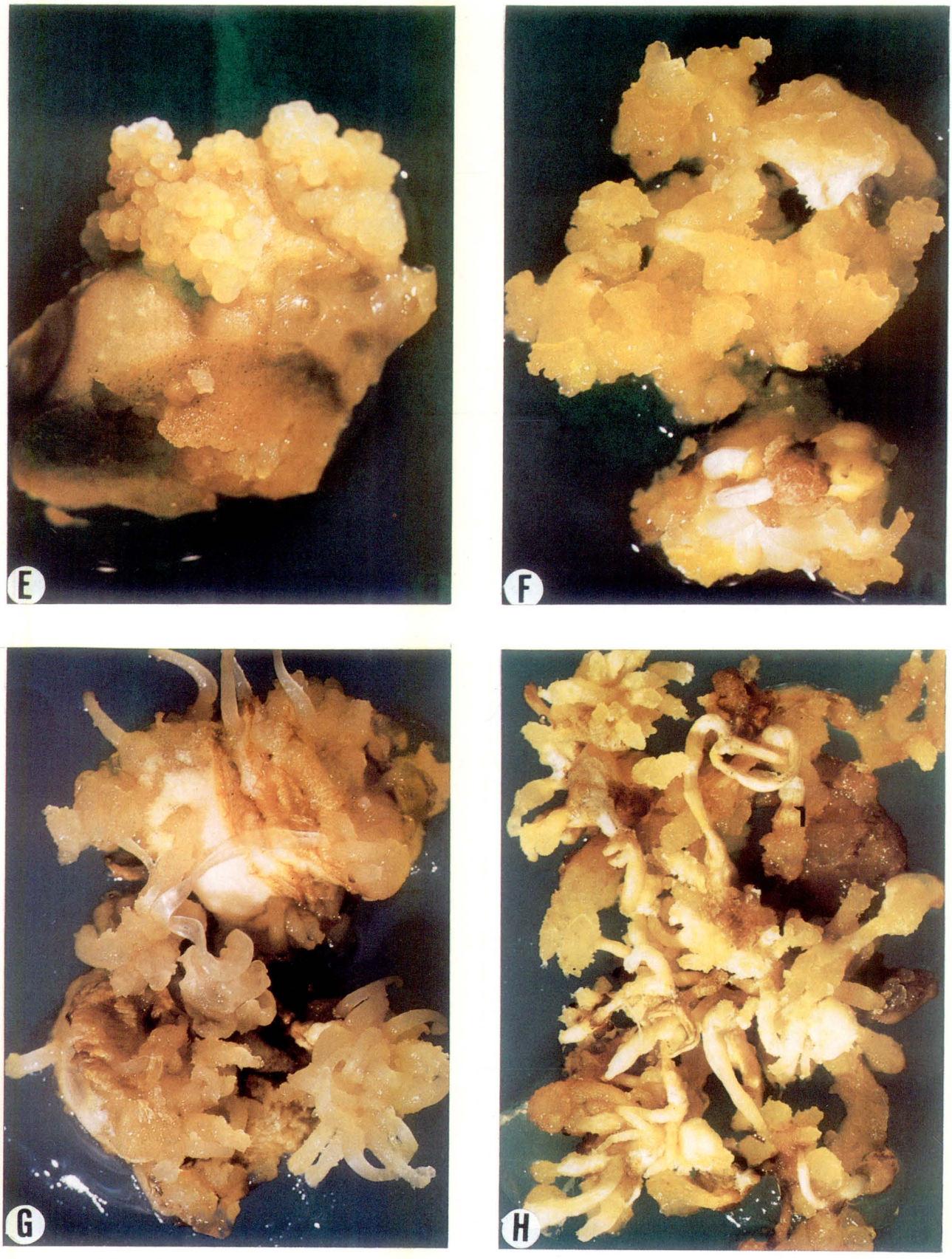


Figure 6



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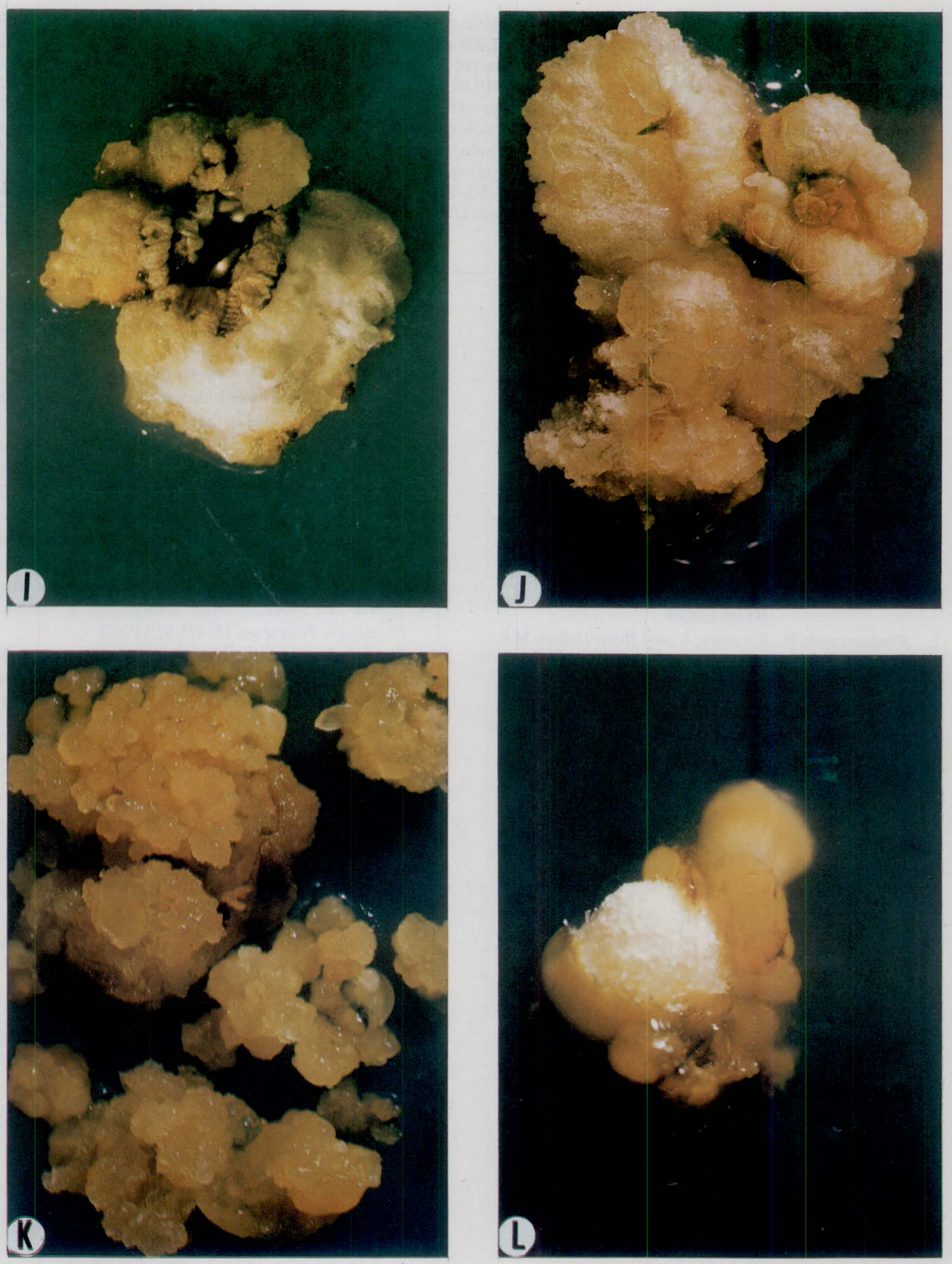

Figure 6 (Continued)

Figure 6. Callus and neoformant organs formation on corm explants. A= Callus formation on corm explant of *C. speciosus* in MS(D0.5-Z0.3) medium after 10 months; B= Callus with ordinary roots and neoformant bud on corm explant of *C. speciosus* in MS(N1-B1) medium after nine months; C= Callus with neoformant bud on corm explant of *C. speciosus* in NS(N5-B1) medium after nine months; D= Callus with neoformant corm on corm explant of *C. speciosus* in MS(N10-B1) medium after a year; E= Callus formation on corm explant of *C. cancellatus* in MS(K0.1-D1) medium after 10 months; F= Callus and contractile roots and corm-like structures on corm explant of *C. cancellatus* in MS(D0.5-Z0.3) medium after 10 months; G= Callus with leaf-like organs and many contractile and ordinary roots on corm explant of *C. cancellatus* in MS(N5-B1) medium after nine months; H= Many contractile roots on corm explant of *C. cancellatus* in MS(N5-B1) medium after a year; I= Callus without organ on corm explant of *C. cancellatus* in MS(N10-B1) medium after nine months; J= Callus without organ on corm explant of *C. almehensis* in MS(N5-B1) medium after nine months; K= Callus with very short, leaf-like organs on corm explant of *C. almehensis* in MS(N10-B1) medium after nine months; L= Callus without organ on corm explant of *C. gilanicus* in MS(K0.1-D1) medium after 10 months

Orchid (Cymbidium) protocorms *in vitro* [5].

Results suggest that the various tissues have specific growth regulator requirements and the trigger for callus formation or organogenesis will only be released when the type and concentration of growth regulator is appropriate. It seems, therefore, that the physiological age of the explant is one of the factors which affects the regeneration response.

Acknowledgements

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