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THE GROWTH OF AND MORPHOGENESIS IN TISSUE CULTURES OF SPINACIA OLERACEA L.

INTRODUCTION

In some plant tissue cultures organ growth can be induced by applying auxins and cytokinins in appropriate ratios, as shown for tobacco tissue (Skoog and Miller, 1957). Steward *et. al.* (1967) pointed to the importance of sequential changes of growth substances, which are necessary for organogenesis not only in some recalcitrant tissues, such as *Asparagus*, but also in many other normal callus cultures. Changes in the pattern of growth, or biochemistry, may also arise spontaneously, probably due to some modifications at the chromosome level, which occur frequently in tissues cultivated for a long time in the presence of growth substances (Gautheret, 1964). If such modifications occur, the composition of the medium may not be regarded as inductive, but rather selective for an especially adapted cell type. This seems to be the case for the organogenesis in spinach tissue, described in this paper. As far as we know, the organogenesis in spinach tissue has not been reported before.

MATERIAL AND METHODS

Seeds of spinach (*Spinacia oleracea* L.) were sterilized by 5% calcium hypochlorite and germinated in sterile vermiculite. The apical parts of the seedlings, about 1 cm long, were cut off and transplanted onto an agar medium. Proliferations arising at the basal parts were further subcultured. The medium contained Murashige and Skoog (1962) mineral solution, 2% sucrose and 1% agar. Further organic constituents of the basal medium (BM) were as follows: Thiamine 3 mg/1, nicotinic acid 5 mg/l, adenine 2 mg/l and kinetin 1mg/l. To this medium were alternatively added: Indole-3-acetic acid 1 mg/1 (designated BM + IAA), 2,4-dichlorophenoxy acetic acid 1 mg/1

(BM + 2,4-D), or gibberellic acid 1 mg/l (BM + GA_3). The cultures were maintained in diffuse light, at about 25°C and subcultured every 6—8 weeks.

RESULTS AND DISCUSSION

Two strains of tissue were grown from the beginning. On the BM + IAA medium the tissue grew in the form of firm, greenish calluses, reaching a fresh weight of 1000—2000 mg per culture period. The tissue had an absolute requirement for both IAA and kinetin. Thiamine, nicotinic acid and adenine were not obligate, but after several transfers without those substances, the growth of the tissue markedly decreased. Although the tissue grows very vigorously, it has not been possible, for more than three years, to induce the growth of organs by any changes in the ratio of IAA to kinetin in the medium, or by supplementing some other organic constituents.

The other strain was grown from the beginning on BM + 2,4-D and produced friable, white calluses. The concentration of 2,4-D was probably supraoptimal, as upon its omission the tissues could still grow during one transfer, but nevertheless died in the next one. However, after almost two years of culture, in a series of calluses transplanted twice on BM + GA_3 all the cultures perished except one callus, which formed a root and small buds and reached a considerable volume after about 5 months. This callus gave rise to a new strain of tissue with a very high bud-forming capacity. When this tissue is returned to the BM + 2,4-D medium, it froms calluses again (Fig. 1a), but reacts very promptly to the omission of 2,4-D by forming roots and buds (Fig. 1b, c, d,). The sequence of BM + 2,4-D $\longrightarrow$ BM + GA_3 $\longrightarrow$ BM + GA_3 $\longrightarrow$ BM + 2,4-D invariably leads to the sequence of callus $\longrightarrow$ roots $\longrightarrow$ shoots $\longrightarrow$ callus. This cycle can repeatedly be induced, no matter of how many times the tissue had been subcultured on either medium.

The buds often develop dark green leaves, with typical adult shape (Fig. 1b). The stems contain the characteristic red pigment of spinach, but the pigment never appears in undifferentiated cells. Occasionally, when the cultures were grown in long days or in the presence of GA_3, female flowers were formed (Fig. 1c).

An investigation of the actual requirements for organogenesis in this strain has shown that the tissue could grow without 2,4-D only if the buds were formed. Small buds obviously supplied a factor necessary for callus growth, the new proliferations produced more buds, and so on. GA_3 does not seem to be essential for bud formation, although they were more abundant in its presence. The omission of kinetin from the BM + GA_3 also decreased the induction of buds.

For more than two years we were trying to repeat the original induction of buds in the callus tissue grown permanently on BM + + 2,4-D, but with no success. If the capacity of forming buds is regarded as a normal feature of the tissue, then it seems that very few cells have retained this feature, or are able to revert into the

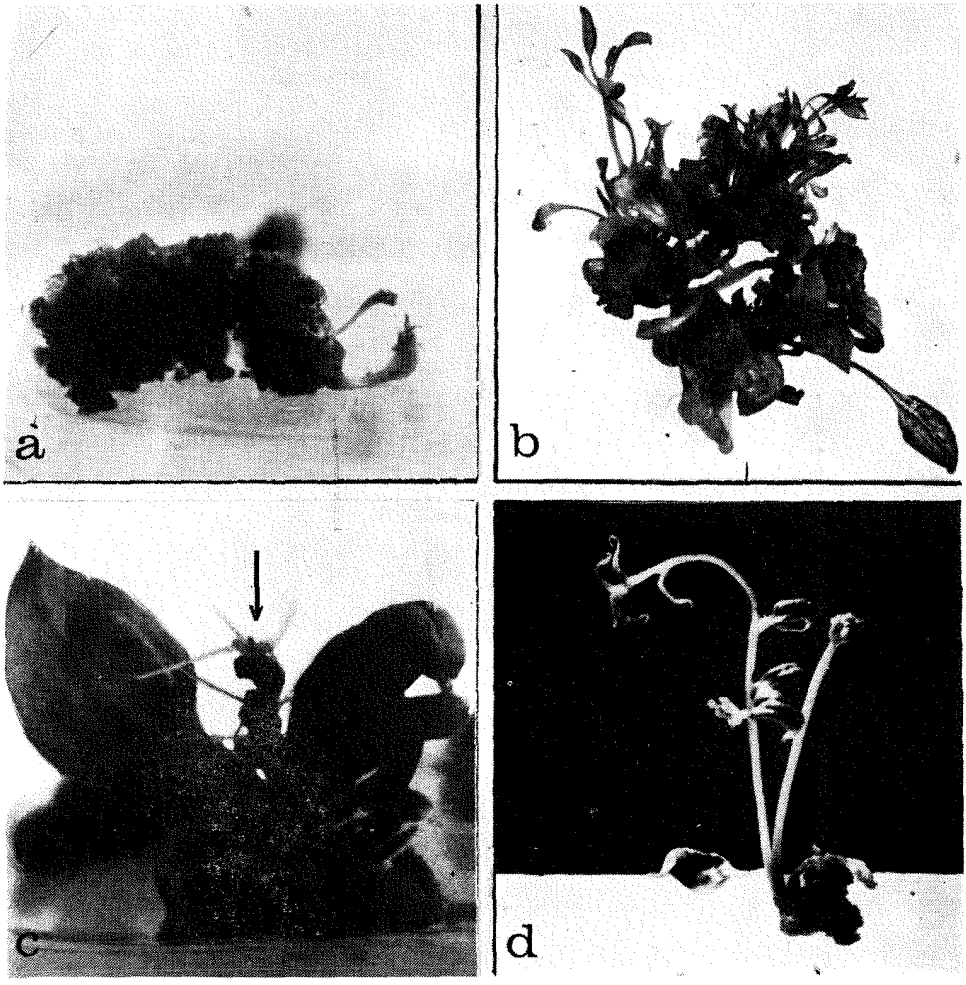

Fig. 1. — Organogenesis in tissue culture of spinach. a: Callus formed after the transfer of tissue from BM + GA_3 to BM + 2,4-D; b, c, d: cultures grown on BM + GA_3; note abundant leaves in b, and a female flower (arrow) in c.

Organogeneza u kulturi tkiva spanaća. a: Kalus obrazovan posle prenosa tkiva sa podloge BM + GA_3 na BM + 2,4-D; b, c, d: tkiva gajena na BM + GA_3; zapaziti dobro razvijene listove na b, kao i ženski cvet (strelica) na slici c.

normal state, after being cultivated on the media with high 2,4-D content. Further study in the cytology of this tissue is in progress.

SUMMARY

An undifferentiated callus culture of *Spinacia oleracea,* grown for a long time on a medium with high 2,4-D content, gave rise to a new strain of tissue possessing very high bud-forming capacity. This strain can be repeatedly induced to grow as callus, or to form organs, by changing the composition of the medium. For organ growth, 2,4-D has to be omitted, while GA_3 and kinetin have a marked stimulatory effect.

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Rezime

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RASTENJE I MORFOGENEZA U KULTURI TKIVA SPINACIA OLERACEA L.

Nediferencirano kalusno tkivo *Spinacia oleracea,* koje je duže vreme kultivisano na podlozi sa visokom koncentracijom 2,4—D, promenilo se i proizvelo kalus od koga je izolovana nova linija tkiva. Novo tkivo ima veoma izraženu sposobnost da formira pupoljke. Ono može naizmenično da raste kao kalusno tkivo ili kao tkivo sa pupoljcima, ako se menja sastav podloge. Da bi se indukovali pupoljci 2,4—D mora da se izostavi, dok GA_3 i kinetin imaju značajan stimulativan efekat.