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INDUCTION OF FLOWERING OF ISOLATED SPINACIA OLERACEA L. BUDS IN STERILE CULTURE

INTRODUCTION

The culture of isolated apical buds presents a convenient method for the study of flowering physiology and biochemistry, which has not been much used so far. This method provides the opportunity to release the apical meristem from the control of the whole organisam and to study its nutritive and hormonal requirements for the onset of reproductive development in strictly controlled environment. In an intact plant it is difficult to know whether an inducting substance or a physical factor affects the apical meristem directly, or through some metabolic change in another, receptor organ. The culture of isolated buds may also suggest whether an interaction exists, in the flowering response, between different plant parts.

Apical meristems of several plant species were isolated and their growth and regeneration studied (Street, 1969; Butenko, 1964). The influence of hormones and photoperiods on the flowering was also studied in some instances (Raghavan, 1961; Raghavan and Jacobs, 1961; Čajlahjan *et. al.*, 1961; Harada, 1967).

The present paper is a preliminary report on the results concerning the isolation and the culture of spinach apical buds. We were interested in the first place to find out whether an isolated bud can be induced to flower under the same conditions as buds in intact plants, which would provide us a basis for further research.

MATERIAL AND METHODS

Seeds of *Spinacia oleracea* L., cv. Matador, were sterilized for 30 minutes by 5% calcium hypoclorite and sown in sterile vermiculite, moistened with Hoagland's mineral solution. The seedlings were grown in short 8 h days. When cotyledons and two paires of leaves were developed, the apical 5 mm of the stems were cut off and transferred onto

different 1% agar media. Mineral solutions of Hoagland, modified by Yoji and Yoshiharu (1964), Heler (1953) and Murashige and Skoog (1962) were tried. Sucrose was added in concentrations 1%, 2% or 3%. Gibberellic acid (GA_3) was either applied to the medium before autoclaving (1 or 10 mg/l), or added to the plants by a micropipette in drops containing 1 or 10 μg per plant. The apices were grown in test tubes with 10 ml or in erlenmayer flasks with 40 ml of the nutrient solution. The cultures were maintained at 25°C, in diffuse light of 1500 lux. The control, non-inductive regime consisted of 8 h-days, while the induction was done by continuous light during 10 days. After 40 days the number of flowering plants was counted and other parameters of growth measured, such as lenght of stems and roots, and the number of internodes, leaves and roots.

RESULTS AND DISCUSSION

The buds transplanted onto agar media showed first signs of growth within 4—5 days, when young leaves start developing. Some apices also developed roots or small calluses at the basal end. The presence of roots was stimulatory to the growth of stems. Plants in short days remained vegetative till the end of experiments, while those in continous light developed flowers (Fig. 1). The absence of roots from most plants did not affect flowering, Of the three mineral solutions tested, Hoagland's solution was superior when the concentration of sucrose was low. With 3% sucrose the growth of the plants was optimal and the mineral solutions had no further effect.

Tab. 1. — Effect of sucrose and gibberllic acid on the vegetative and reproductive development of isolated buds in short days.

Nutritive medium*	Number of			Lenght (mm)		% of
	Cultures	Leaves	Flowers	Stems	Roots	Flowering
Sucrose 1%						
GA_3 1 μg/plant	13	6	4	21	42	30
10 "	19	4	4	21	31	21
GA_3 1 mg/l	22	4	8	33	22	36
10 "	11	4	2	36	14	18
Sucrose 3%						
GA_3 1 mg/l	9	6	4	67	27	44
10 "	10	4	3	47	20	33

* Hoagland's mineral solution, 1% agar.

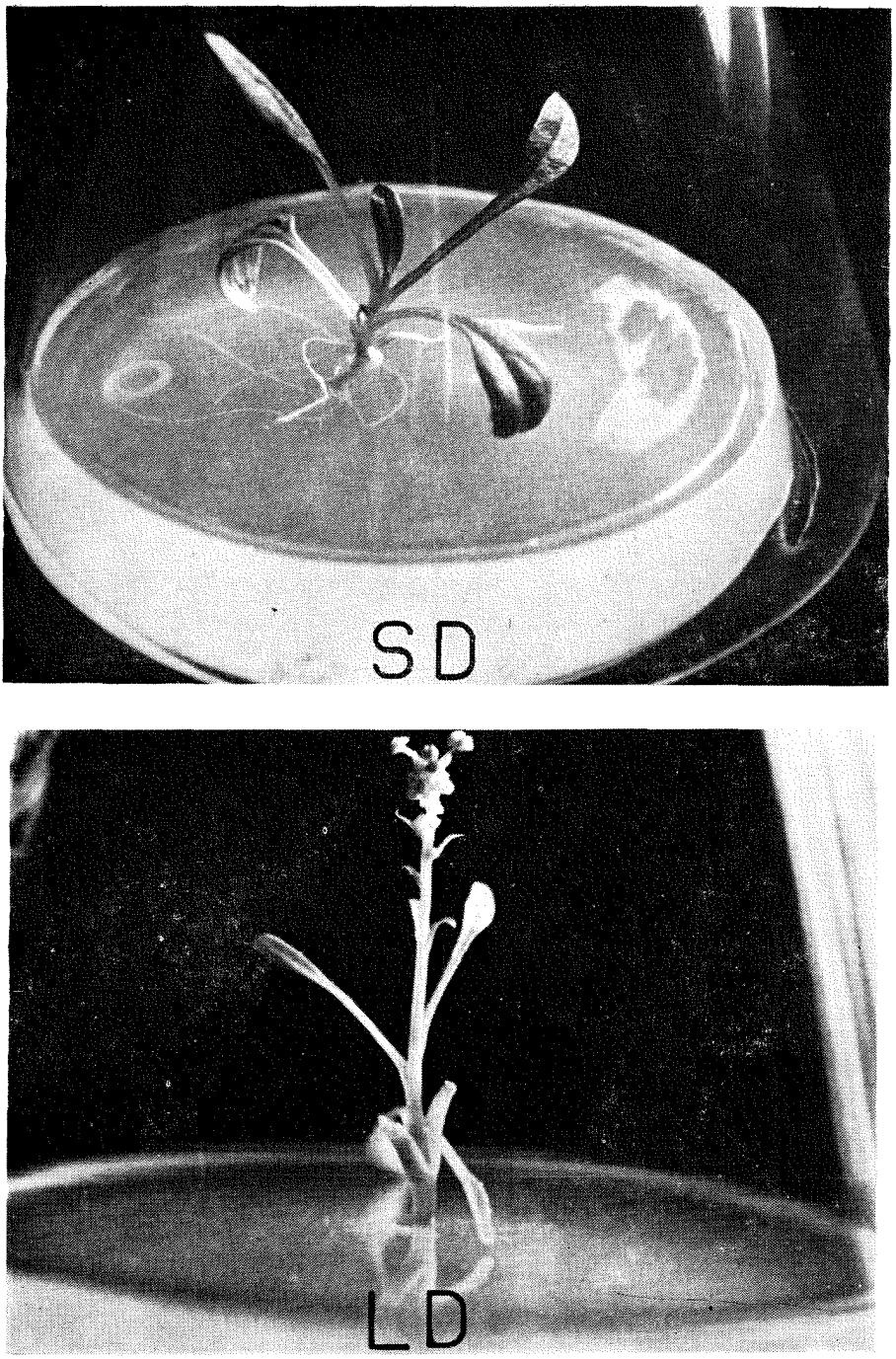

Fig. 1. — Isolated apical buds grown in culture for 40 days. SD — vegetative bud in short days of 8 h. LD — flowering buds induced by continuous light.
Isolovani apikalni pupoljci gajeni u kulturi 40 dana. SD — vegetativni pupoljak na kratkom danu od 8 h. LD — pupoljak koji cveta indukovan kontinualnom svetlošću.

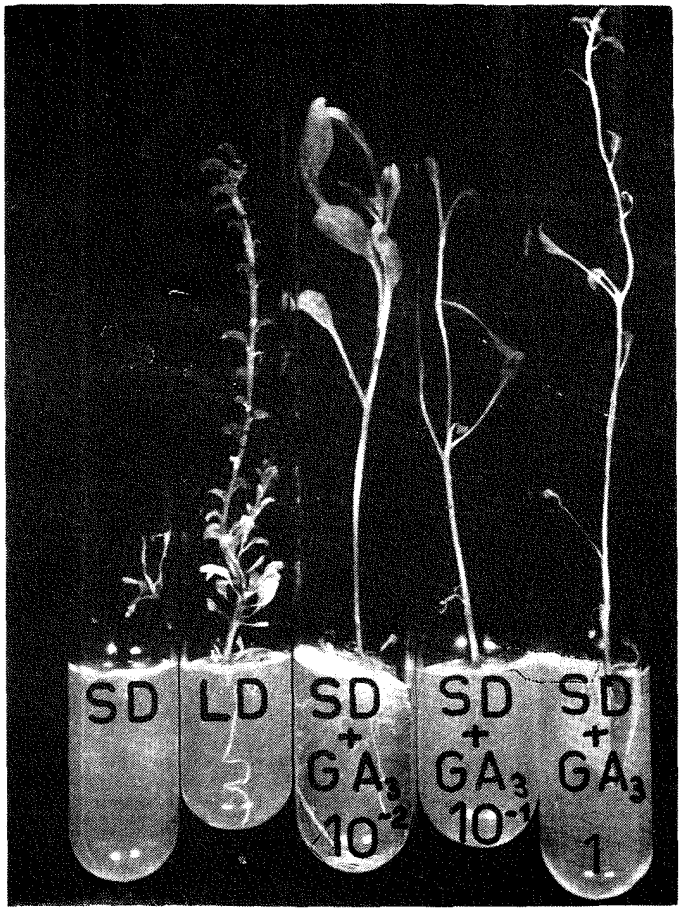

Fig. 2. — Flowering bud grown in short days (8 h) and treated with GA_3 (1 mg/l in the medium).

Pupoljak koji cveta gajen na kratkom danu (8 h) uz dodavanje GA_3 (1 mg/l u medijum).

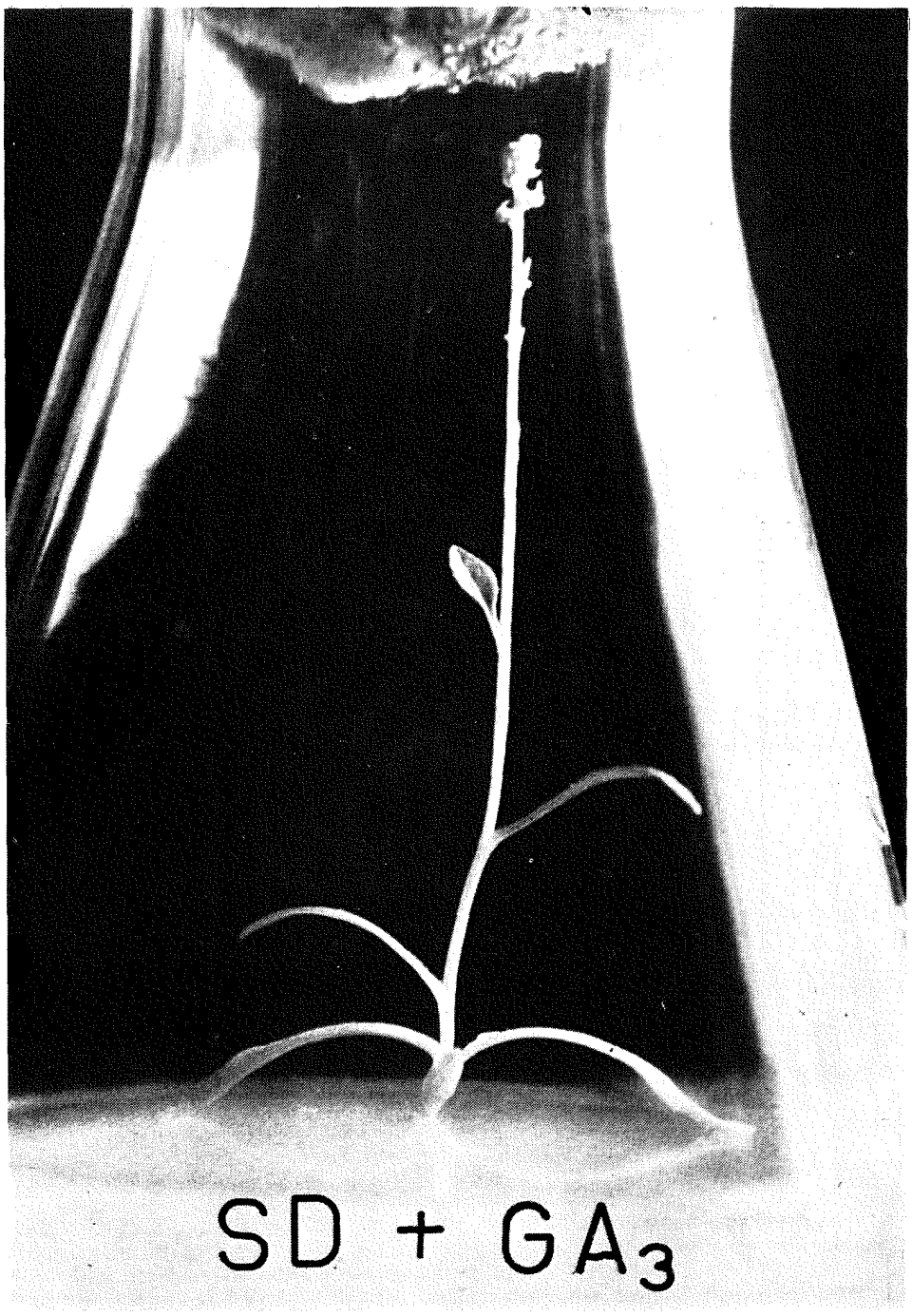

Fig. 3. — Buds grown in mineral solution of Murashige and Skoog, 3% sucrose and GA_3 (10^{-2}, 10^{-1} and 1 mg/l), in short (SD) and long (LD) days. Note the opposite effect of sucrose and GA_3 on the lenght of internodes.

Pupoljci gajeni na mineralnom rastvoru Murashige i Skoog, 3% saharozi i GA_3 (10^{-2}, 10^{-1} i 1 mg/l), na kratkom (SD) i dugom (LD) danu. Treba zapaziti suprotan efekat saharoze i GA_3 na dužinu internodija.

Rezime

LJUBINKA ĆULAFIĆ

INDUKCIJA CVETANJA APIKALNIH PUPOLJAKA SPINACIA OLERACEA U KULTURI IN VITRO

U radu Ćulafić i Nešković (1974) utvrđene su osnovne karakteristike fotoperiodskog režima za biljku dugog dana *Spinacia oleracea,* sorta „Matador", gajenu na vermikulitu sa Hoaglandovim mineralnim rastvorom. Kritična dužina dana je dvanaest časova. Režim od osam časova svetlosti i šesnaest časova mraka je neinduktivan. Pet kontinualnih ciklusa osvetljavanja predstavljaju prag indukacije i izazivaju 59% cvetanja, a osam kontinualnih ciklusa 95% cvetanja. Fotoperiodsku indukaciju moguće je zameniti dodavanjem giberelina u koncentraciji 1 i 10 μg na biljku ili u medijum.

Kako je poznato da sistem list-apikalni pupoljak ostvaruje fotoperiodsku indukaciju, mi smo proveravali da li izolovani apikalni pupoljak sa najmlađim listovima može cvetati pod istim fotoperiodskim režimom kao i intaktna biljka, koji mineralni rastvor je najpovoljniji za cvetanje, kako ovaj sistem odgovara na egzogeno dodavanje GA_3 i dodavanje GA_3 u medijum. Eksperimenti su pokazali da 44% apikalnih pupoljaka u kulturi in vitro cveta na Hoaglandovom mineralnom rastovru 3% saharozi i koncentraciji od 1 μg GA_3 dodatog u medijum.

Značajno je zapaziti da su mladi listovi u stanju da prime fotoperiodsku indukaciju i da apikalni pupoljak cveta i razvija morfološki potpuno normalne muške i ženske cvetove.

Ovako izolovani sistem, u pogledu odgovora na fotoperiodsku indukaciju, adekvatan je intaktnoj biljci a osetljiviji je na GA_3 od intaktne biljke pa je zato pogodniji za ispitivanje uticaja različitih faktora na cvetanje.