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Short communication

Spermidine and flower-bud differentiation in thin-layer explants of tobacco

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Abstract. Three lines of evidence indicate a connection between high spermidine levels and floral initiation in thin-layer tissue cultures of Wisconsin-38 tobacco (*Nicotiana tabacum* L.). (1) Spermidine levels are much higher in floral buds than in vegetative buds. (2) Inhibition of spermidine synthesis by cyclohexylamine prevents the rise in spermidine titer, inhibits floral initiation and promotes the formation of vegetative buds instead. (3) Application of exogenous spermidine causes floral initiation in cultures which would otherwise form vegetative buds.

Key words: Flower initiation – *Nicotiana* (flower differentiation) – Polyamine – Spermidine – Tissue culture (thin layer).

The internal events regulating floral initiation remain obscure, despite a half century of intensive work and strong evidence for the movement of a florigenic stimulus from leaves to buds in photoperiodically induced plants (for a review, see Vince-Prue, 1975). In particular species, auxin, cytokinin and gibberellin can induce flowering, but none of these substances satisfies the requirements for the hypothetical flowering hormone, which is believed to be effective in both long-day and short-day plants and transmissible between them.

Recent studies indicate a possible role of polyamines, especially spermidine, in reproductive differentiation in various organisms. These include regulation of sporulation in yeast and various filamentous fungi (Tabor and Tabor 1985; Rajam and Galston 1985; Birecka et al. 1986), differentiation of embryoids in tissue cultures of carrot (Feirer et al. 1984, 1985) and *Vigna* (Kaur-Sawhney et al. 1985), the abnormal flowering habits of polyamine mutants of tobacco (Malmberg and McIndoo 1983) and the accumulation of acylated polyamines in flowering, but not vegetative tobacco plants (Martin-Tanguy 1985). Spermidine is biologically ubiquitous, and has also been implicated in regulation of cell growth and development in plants and animals (reviewed in Heby 1981; Slocum et al. 1984). Spermidine synthesis from the diamine putrescine is controlled by spermidine synthase, which transfers an aminopropyl group from S-adenosylmethionine to one terminal amino group of putrescine (Slocum et al. 1984).

In this paper we report a possible role of spermidine in floral bud initiation in thin-layer tobacco tissue cultures (Tran Thanh Van 1973a, b).

Explants were obtained by removing thin strips of surface tissue from the internodes of floral branches at the green fruit stage of Wisconsin-38 tobacco plants (*Nicotiana tabacum* L.) grown as described in Tiburcio et al. (1985). Free and conjugated polyamines were extracted, dansylated, solvent-purified, separated by thin-layer chromatography (TLC) and quantified using an Aminco-Bowman spectrophotofluorimeter (American Instruments Co., Silver Spring, Md., USA), as detailed in Tiburcio et al. (1985). Protein in the insoluble PCA-pellet resuspended in 1 N NaOH was determined according to the method of Bradford (1976), with bovine γ-globulin (Sigma Chemical Co., St. Louis, Mo., USA) as a standard.

On an agar-solidified Murashige-Skoog (1962) medium supplemented by sucrose, α-naphthaleneacetic acid (NAA) and kinetin (N^6-furfurylaminopurine), explants consisting of epidermis and two to five subepidermal cell layers initiated cell division within 2 d and differentiated obviously vege-

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Table 1. Polyamine levels in floral and vegetative buds formed from thin-layer explants of tobacco. Explants were grown on Murashige-Skoog medium containing 1 μM NAA and 1 μM kinetin for formation of floral buds, and 10-fold higher kinetin for vegetative buds. Titers are the sum of free and conjugated forms. Experiments done twice, with three replicates in each. Data represent means ± SE

Age of culture (d)	Putrescine (nmol·(mg protein)$^{-1}$)			Spermidine (nmol·(mg protein)$^{-1}$)		
	Vegetative (V)	Floral (F)	F/V	Vegetative (V)	Floral (F)	F/V
0	88 ± 9			83 ± 12		
9	249 ± 29	167 ± 15	0.63	201 ± 24	241 ± 1	1.19
15	331 ± 42	227 ± 24	0.68	189 ± 22	255 ± 17	1.35
20	769 ± 59	834 ± 62	1.09	217 ± 21	436 ± 73	1.96
28	879 ± 84	1128 ± 69	1.28	188 ± 20	865 ± 20	4.54

tative and floral buds after 9 and 15 d, respectively. When NAA and kinetin concentrations were both 1 μM, about 94% of the buds were floral; raising the cytokinin concentration to 10 μM caused a change in the differentiation pattern to 100% vegetative buds.

In explants regenerating either vegetative or floral buds, the titers of putrescine and spermidine rose sharply above initial values (Table 1). At the time of vegetative bud determination, putrescine was the dominant polyamine, while in floral buds, spermidine predominated. By day 28 after explantation, the spermidine titer in floral buds was 4.54 times higher than in vegetative buds, while the comparable ratio for putrescine was only 1.28.

Table 2. Effect of cyclohexylamine on spermidine titer and floral differentiation in thin-layer explants of tobacco. Explants were grown on medium containing 1 μM each of NAA and kinetin, in which more than 94% of the buds are floral. Cyclohexylamine was added to the medium at the start of culture. The spermidine titer (± SE) and the nature of the buds were determined 28 d later

Cyclohexylamine (mM)	Spermidine titer (nmol·(g FW)$^{-1}$)	No. buds/12 explants	
		Floral	Vegetative
0	841 ± 93	21	0
2	530 ± 174	12	4
10	388 ± 71	5	8
20	216 ± 30	2	31

To probe the possible importance of high spermidine titer in the differentiation of floral buds, we cultured thin-layer explants in the presence of cyclohexylamine, a competitive and reversible inhibitor of spermidine synthase (Sindhu and Cohen 1984; Batchelor et al. 1986). Treatment with cyclo-

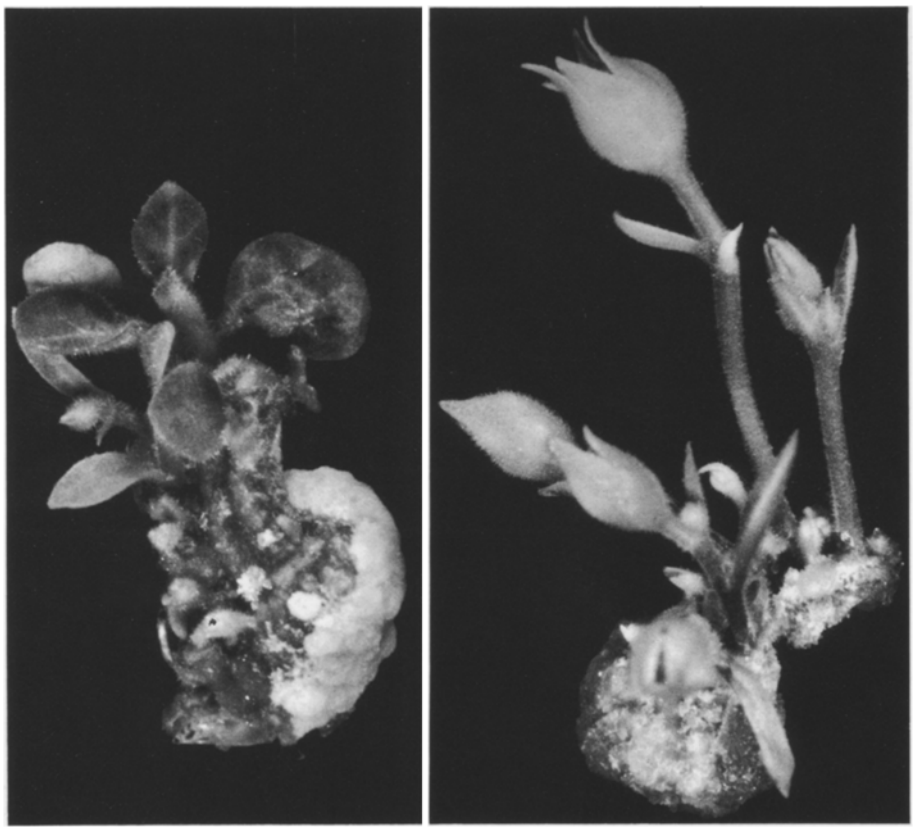

Fig. 1. Effect of spermidine on bud differentiation in thin-layer explants of tobacco. Explants were cultured in the presence (*right*) or absence (*left*) of 5 mM spermidine. Photographs were taken when the cultures were one month old and buds fully developed. × 2.5

Table 3. Effect of exogenous spermidine on bud differentiation in thin-layer explants of tobacco grown on medium containing 1 μM NAA and 10 μM kinetin, which causes differentiation of 100% vegetative buds. The formation of floral and vegetative buds was scored after one month of culture. Experiment repeated six times. Figures represent means of two experiments with four replicates in each treatment

Spermidine conc. (mM)	No. buds/16 explants		
	Vegetative	Floral	Total
0	403	0	403
0.5	144	55	199
1.0	144	37	181
5.0	103	35	138

hexylamine inhibited the differentiation of floral buds in a concentration-dependent manner (Table 2), and such inhibition was correlated with a switch to the initiation of vegetative instead of flower buds. Addition of 1 mM spermidine to cultures inhibited with 10 mM cyclohexylamine produced partial reversal, in that it prevented the appearance of any vegetative buds while only slightly reducing the number of flower buds.

A direct effect of spermidine on the differentiation of floral buds is shown by adding exogenous spermidine to explants growing on "vegetative" medium (Fig. 1). Such cultures develop more than 20% floral buds, rather than 100% vegetative buds, irrespective of the concentration of spermidine added (Table 3). Floral differentiation is correlated with a decline in the total number of buds formed (Table 3), probably reflecting the larger size and "sink" effect of developing flower buds.

Do these observations have any bearing on the general problem of flowering? We recognize that this in-vitro tobacco system may beg the question of induction to flower because the thin layers are taken from plants that are already flowering. To obtain vegetative buds from the explants, we must mask the flowering tendency by a high (10 μM) cytokinin level in the medium. Thus, the florigenic action of spermidine in this system may result merely from its negation of the effect of high cytokinin levels. To obtain further relevant information, we are now studing the effects of photoperiodic induction on polyamine metabolism in short- and long-day plants.

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