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IN VITRO CULTURES OF YOUNG STAMENS OF *TRADESCANTIA**

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ABSTRACT

Young stamens of *Tradescantia* clone 02 were cultured in vitro on agar media containing various combinations of three plant hormones, IAA, GA_3 and kinetin. Growth and coloration of stamen hairs proved to be controlled by some hormones. The maximum growth of stamen hairs was observed when cultured on the medium which contained 5.0 mg/*l* IAA, 1.0 mg/*l* GA_3 and 01 mg/*l* kinetin. As much as a 10-cell increase was observed in stamen hairs in this case, although the growth observed did not reach completely the normal growth *in vivo*. The hormonal concentrations of the medium which induced the maximum coloration were 0.1 mg/*l* IAA, 1.0 mg/*l* GA_3 and 0.1 mg/*l* kinetin. Higher concentration of sucrose also seemed to be effective in promoting coloration.

The pattern of coloration indicated the existence of some trigger(s) to coloration, and hormones were considered to have some effects on the trigger (s). The distribution pattern of colored cells along hairs (more coloration at hair tips) supported the idea that completion of cell divisions is one of the triggers of the coloration.

The present study proved the possibility of developing *in vitro culture* techniques of *Tradescantia* stamens.

RESUMEN

Jóvenes estambres del clone 02 de *Tradescantia* fueron cultivados *in vitro* en medio de agar que contuvo diversas combinaciones de tres hormonas vegetales: IAA, GA_3 y kinetina.

Se demostró que tanto el crecimiento como la coloración de los pelos estaminales fueron controlados por algunas hormonas. El máximo crecimiento de los pelos estaminales se observó cuando se cultivaron en medio que tuvo 5.0 mg/*l* de IAA, 1.0 mg/*l* de GA_3 y 0.1 mg/*l* de kinetina, en cuyo caso un incremento de 10 células se notó en los pelos estaminales aunque el crecimiento no alcanzó totalmente el que se desarrolla *in vivo*. Las concentraciones hormonales del medio que indujeron la coloración máxima fueron 0.1 mg/*l* de kinetina. Mayores concentraciones de sacarosa también fueron efectivas en promover la coloración.

El molde de coloración indicó la existencia de algún(os) mecanismo(s) de disparo de la coloración y se consideró que las hormonas estaban involucradas en ello. El esquema de distribución de las células coloreadas, a lo largo de los pelos (mayor coloración en las puntas) apoyó la idea de que la terminación de las divisiones celulares es uno de los disparos de la coloración.

En este trabajo se probó la posibilidad de desarrollar las técnicas de cultivo *in vitro* de los pelos estaminales de *Tradescantia*.

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Abbreviations: IAA, indole-3-acetic acid; GA_3, gibberellic acid.

INTRODUCTION

It is widely known that *Tradescantia* species have features particularly well situed for certain radiobiological studies (see Ichikawa and Sparrow, 1967, 1968; Ichikawa *et. al.*, 1969). Especially, the stamen hairs of some clone of *Tradescantia* have proved to be useful for studying induced mutation rates at low levels of radiation doses in the millirad region (Ichikawa, 1971, 1972; Sparrow *et al.*, 1972). The techniques for using the stamen-hair system have been developed over the past decade especially for radiobiological studies. However, it has been required to apply these techniques to experiments with known chemical mutagens and some other substances which exist in our environment (Underbrink *et al.*, 1973; Ichikawa, 1976).

Sparrow and Schairer (1974) and Sparrow *et al.* (1975) treated *Tradescantia* stamen hairs with some chemicals in gaseous form. Ichikawa (1976) and Ichikawa and Takahashi (1978) developed a method to apply chemical mutagens as aqueous solutions. With these methods, however, the concentrations of mutagens which actually penetrated into the tissue remained uncertain, and thus, it is desirable to develop a new method with which young stamen hairs can be exposed to chemical mutagens more directly.

In the present study, the authors tried to depelop *in vitro* culture techniques of the stamens of *Tradescantia*, as such a new method applirable for mutagenicity testing of chemicals.

MATERIAL AND METHODS

The material used in the present study was *Tradescantia* clone 02 (BNL 02), a diploid ($2n = 12$) hybrid clone heterozygous for flower color (blue/pink, the blue being dominant). The origin of this clone is not clear (see Mericle and Mericle, 1967), but the characteristics of the clone were described elsewhere Nayar and Sparrow, 1967; Mericle and Mericle, 1967; Ichikawa *et al.*, 1969). This clone, originally supplied by late Dr. A. H. Sparrow of Brookhaven National Laboratory of USA in 1974, had been maintained in the airconditioned greenhouse of the Laboratory of Genetics of Kyoto University. The temperature in the greenhouse was kept between about 18 and 23°C, and the day-length was adjusted to be 17 hrs throughout the experimental period.

Flower buds of 2 to 4 mm in length were excised from young inflorescences of potted plants grown in the greenhouse. Excised buds were dipped into 10% solution of sodium hypochloride to prevent microbial contamination then washed with sterile water three times. After removing sepals and petals and excising one out of six stamens (one antipetalous stamen, see below) of each bud under stereoscope, the remaining part (composed of five stamens plus ovary) was planted on one of the agar media described below. In most cases, 30 buds were used for each medium.

Basal medium used was White's medium without $CuSO_4.H_2O$ and MoO_3. Three plant hormones (IAA, GA_3 and kinetin) of 11 different combinations were added to the basal medium in the first experiment as shown in Table I, and three out of

the 11 combinations were re-examined in the second experiment. Also added were 0.5% agar and 4% sucrose, except for one medium to which 8% sucrose was added (see the footnote of Table I).

The excised stamen was employed for determining the extent of hair growth in each bud at the time of planting. For this purpose, the number of hair cells was counted for the basal six hairs of each excised stamen, and the data collected were used for comparing with similar data to be taken after culturing.

After 10-day culturing at 21, 23 or 26°C (see Table I) being illuminated continuously at a light intensity of about 4000 lx, all the stamens were harvested and the hair cell numbers of six basal hairs were counted to know the hair growth during the cultures. The coloration of hair cells was also recorded for all hairs.

TABLE I

CONCENTRATIONS OF IAA, GA_3 AND KINETIN SUPPLEMENTED, EXPERIMENTAL PERIODS, AND TEMPERATURE DATA.

Medium no.	*Concentration (mg/l)*			*Experimental period*	*Temperature (°C)*	
	IAA	*GA_3*	*Kinetin*		*Stock plants grown at*	*Cultured at*
Experiment 1						
1	0.1	1.0	0.1	March, 76	20 ± 2	23 ± 1
2	1.0	1.0	0.1	„	„	„
3	5.0	1.0	0.1	„	„	„
4	0.1	1.0	0.05	August, 76	23 ± 1	26 ± 1
5	0.1	0.6	0.05	„	„	„
6	0.1	5.0	0.1	„	„	„
7	0	1.0	0.05	November, 76	18 ± 2	21 ± 1
8	0	5.0	0.1	„	„	„
9	0.1	5.0	0.2	„	„	„
10	0.1	5.0	0.5	„	„	„
11	0.1	10.0	0.5	„	„	„
Experiment 2						
R1	(the same as medium 1)			January, 77	20 ± 3	21 ± 1
R3	(the same as medium 3)			„	„	„
R5	(the same as medium 5)			„	„	„
M1*	(the same as medium 1)			„	„	„
0	0	0	0	„	„	„

* Contained 8% sucrose. Other media contained 4% sucrose.

RESULTS

Experiment 1

The growths of hairs occurred during the cultures on the media 1 to 11 used in Experiment 1 (see Table I) are presented in Table II. The growth was expressed by the increase of cell number per hair during each culture, which was obtained by

subtracting the average number of cells per hair at the time of starting culture (determined with the excised antipetalous stamen) from the average number of cells per hair after culture (determined with the two antipetalous and three antisepalous stamens cultured) for each bud. In order to analyze the extent of the hair growth precisely, the buds cultured were classified into the following four classes according to the average number of cells per hair in the stamen examined before culture for each bud; namely, average cell number per hair was less than 10.0 (I), between 10.0 and 14.9 (II), between 15.0 and 19.9 (III), and 20.0 or more (IV). The data of hair growth obtained are classified into the four classes in Table II.

TABLE II

THE GROWTH (INCREASE IN CELL NUMBER) OF FOUR CLASSES OF STAMENS (SEE TEXT) AFTER 10-DAY CULTURING ON 11 DIFFERENT MEDIA CONTAINING VARIOUS CONCENTRATIONS OF IAA, GA_3 AND KINETIN (SEE TABLE I FOR THE HORMONE CONCENTRATIONS).

Medium no.	*Class of stamens**	*Cell number/hair (± SD)*		
		Before culture	*After culture*	*Growth*
1	I	——	——	——
	II	13.2 ± 0.7	15.3 ± 2.0	2.1 ± 1.6
	III	18.2 ± 1.3	20.9 ± 2.0	2.7 ± 1.5
	IV	22.9 ± 1.6	23.1 ± 1.1	0.2 ± 1.2
2	I	8.2 ± 1.4	14.3 ± 0.9	6.0 ± 1.5
	II	13.0 ± 1.0	17.5 ± 2.0	4.6 ± 1.6
	III	17.9 ± 0.9	20.0 ± 1.9	2.2 ± 1.2
	IV	21.0 ± 0.9	21.4 ± 1.4	—0.5 ± 1.7**
3	I	8.6 ± 1.1	18.6 ± 2.9	10.0 ± 3.0
	II	12.4 ± 1.7	17.8 ± 2.4	5.4 ± 3.4
	III	17.0 ± 1.2	18.2 ± 2.6	1.3 ± 3.2
	IV	21.8	22.5	0.7
4	I	7.6 ± 1.5	13.3 ± 0.6	5.7 ± 2.0
	II	12.6 ± 1.2	16.0 ± 2.5	3.5 ± 1.9
	III	17.1 ± 1.6	19.1 ± 1.6	2.0 ± 1.8
	IV	21.9 ± 1.8	21.4 ± 1.4	—0.5 ± 1.3**
5	I	7.8 ± 1.5	10.7 ± 2.2	2.9 ± 1.9
	II	12.5 ± 1.5	15.4 ± 1.8	2.9 ± 1.8
	III	17.0 ± 0.9	17.7 ± 1.2	0.6 ± 0.6
	IV	——	——	——
6	I	——	——	——
	II	13.4 ± 1.0	17.2 ± 1.1	3.8 ± 1.3
	III	17.6 ± 1.5	19.7 ± 1.4	2.1 ± 1.6
	IV	21.4 ± 0.6	21.7 ± 0.5	0.3 ± 0.6
7	I	7.6 ± 2.0	10.0 ± 1.8	2.3 ± 2.5
	II	12.8 ± 1.3	14.8 ± 1.6	2.0 ± 1.4
	III	16.8 ± 1.2	17.0 ± 1.4	0.3 ± 1.2
	IV	——	——	——

8	I	6.8 ± 2.0	9.2 ± 2.2	2.3 ± 1.2
	II	11.3 ± 1.3	12.9 ± 1.7	1.6 ± 1.3
	III	17.3 ± 1.5	18.6 ± 2.0	1.4 ± 1.4
	IV	22.0	22.5	0.5
9	I	6.6 ± 2.1	10.1 ± 2.6	3.5 ± 1.5
	II	10.9 ± 0.7	14.3 ± 1.8	3.3 ± 1.5
	III	15.3	19.6	4.3***
	IV	——	——	——
10	I	7.3 ± 1.7	11.1 ± 2.2	3.8 ± 1.1
	II	12.3 ± 1.7	13.8 ± 0.5	1.5 ± 1.3
	III	17.3	17.7	0.4
	IV	——	——	——
11	I	8.4 ± 1.6	10.8 ± 2.2	2.4 ± 1.6
	II	12.1 ± 0.8	14.4 ± 1.4	2.3 ± 1.6
	III	16.5 ± 0.8	17.7 ± 0.7	1.2 ± 0.7
	IV	——	——	——

* See text.

** When growth is little, sampling errors may exceed the growth (scorings were made on six basal hairs of an antipetalous stamen before culture, and on basal six hairs each for two antipetalous and three antisepalous stamen after culture; the antipetalous stamen tend to have longer hairs during hair development stage).

*** The value shows an excellent growth, but from only single material.

For the class I stamens which should potentially have the greatest growing ability of hairs, the largest hair growth was observed with the medium 3, and relatively good growths were also observed with the media 2 and 4. For the class II stamens also, the medium 3 resulted in the largest growth and the media 2 and 4, as well as the medium 6 (for which no datum was available for class I), showed good growth. For the class III stamens which should have much less potential ability of further hair growth, no very clear conclusion might be made, but the media 1, 2, 4 and 6 seemed to be more effective than others in promoting hair growth. Only slight hair growths, if any, were observed for the class IV stamens as expected (in this class, further growth of hairs must be small because of being closer to the end of the growth).

For elucidating the interactions of hormones or the effect of each hormone under influences of other hormones, several combinations of comparisons of hair growth could be made between different media. The effect of IAA under the existence of constant amounts of GA_3 and kinetin can be determined when such comparisons are made between the media 1, 2 and 3, between 4 and 7, or between 6 and 8 (see Table I). In these comparisons, it is clear that growth of stamen hairs was largest with higher concentrations of IAA, when the concentrations of GA_3 and kinetin were kept unchanged (see Table II). Likewise, from the comparisons between the media 4 and 5 and between 10 and 11, it was apparent that growth of stamen hairs was inversely correlated to the concentration of GA_3 when the concentration of IAA was 0.1 mg/*l* and that of kinetin was 0.05 or 0.5 mg/*l* (see Tables I and II). No clear relationship was found, however, from the comparison of the media 1 and 6 in which the concentrations of both IAA and kinetin

were 0.1 mg/*l*, but these cases might be affected by the different culture conditions (see Table I). Concerning the effect of kinetin, it was expected that comparisons between the media 1 and 4 and between 5, 6, 9 and 10 would give informations, but any clear effect of kinetin was not found (see Tables I and II).

TABLE III

FREQUENCY OF COLORATION OCCURRED IN STAMEN HAIRS AFTER 10-DAY CULTURING ON 11 DIFFERENT MEDIA CONTAINING VARIOUS CONCENTRATIONS OF IAA, GA_3 AND KINETIN (SEE TABLE I FOR THE HORMONE CONCENTRATIONS).

*Medium no.**	*Number of colored stamen hairs per flower***			
	Class I	*Class II*	*Class III*	*Class IV*
2	—	21.0	31.9	12.3
2	4.4	10.9	8.7	2.5
3	5.5	2.2	1.3	0
9	0	0	2.0	—

* No coloration occurred on other media not listed in this table.
** "Flower" means a cluster of five stamens plus ovary cultured. All the five stamens did not always survive.

The frequencies of coloration occurred in the stamen hairs after cultures are shown in Table III. The coloration occurred only with four media (the media 1, 2, 3 and 9) out of the 11 different media tested. The coloration of stamen hair cells occurred most frequently with the medium 1. From the comparison of the results on the media 1, 2 and 3, lower IAA concentration seemed more suitable for the coloration of hairs.

TABLE IV

DISTRIBUTION OF COLORED CELLS IN THE STAMEN HAIRS AFTER CULTURING ON THE MEDIA 1, 2 AND 3.

Order of cells from hair tip	*Number of cells colored on*			*Total*
	Medium 1	*Midum 2*	*Medium 3*	
Terminal	181	83	18	282
Subterminal	243	68	24	335
3rd	105	32	15	152
4th	92	23	13	128
5th	39	11	8	58
6th	27	4	6	37
7th	6	2	3	11
8th	5	2	3	10
9th	0	0	1	1
10th	3	0	0	3
Total number of hairs examined	471	169	58	698

The distributions of colored cells along the stamen hairs are presented in Table IV for the media 1, 2 and 3. No essential difference in the distributions was found among the three media. It is seen that the subterminal cells colored most frequently, the terminal cells next frequently, and the frequency decreased steadily from the third through tenth cells from the hair tip (also see Fig. 4).

Experiment 2

The results of Experiment 1 showed that the coloration of stamen hairs was caused under certain hormonal conditions. However, there were great individual differences between inflorescences and between flower buds. To ascertain those results, it is therefore thought necessary to re-examine some of the media used in Experiment 1. Re-examined were the media 1, 3 and 5, and they were designated as the media R1, R3 and R5, respectively, in Experiment 2. Also, as an aid to know the hormonal effects more exactly, a medium with addition of only agar and sucrose (no plant hormone added) to the basal medium was examined in this experiment, and it was designated here as the medium 0. Furthermore, in order to know the effect of sucrose concentration, a modified medium 1 containing 8% sucrose instead of 4% was examined designating it as the medium M1 (see Table I). In this second experiment, culturing the stamens of class IV was not tried since they were proved in Experiment 1 to have very little potential ability of further growth. Thus, the stamens of classes I, II and III were exclusively employed in this experiment.

The data of the growth of stamen hairs during cultures on these five media (R1, R3, R5, M1 and 0) are presented in Table V. As seen in this table, relatively good growths were observed on the media R1, R5 and M1 rather than on the medium R3 on which (the medium 3 in Experiment I) the maximum growth had been observed in the former experiment. The cause of the discrepancy remains unknown, and these results must be re-examined.

It was found that stamen hairs seemed to grow to some extent on the medium 0, although it may not be said confidently that they grew because of great standard deviations in comparison with the mean values for the growth (see Table V). Further examination is also required for this aspect.

The data of coloration of hair cells are shown in Table VI. The medium M1 resulted in the best coloration, far exceeding other media. Comparing this result with those from the medium R1 in this experiment and the medium 1 in Experiment 1, it seems possible to say that the coloration is promoted by sucrose. However, other concentrations of sucrose must be examined before giving any definitive conclusion. Coloration occurred on the medium R5 in this experiment (the extent of coloration was comparable with or higher than the medium R1), showing a sharp contrast with no coloration at all on the medium 5 (the same medium as R5) in Experiment 1 (see Table III). One may suspect that the culture conditions in Experiment 2 were more suitable for coloration than those in Experiment 1, but it does not seem to stand because the frequencies of coloration on the medium R1 were apparently lower than those on the medium 1

(see Tables III and VI). Thus, some unknown factor(s) might be concerned in the color expression. Any coloration was not observed on the medium 0, proving that hormones are required for the coloration.

TABLE V

THE GROWTH (INCREASE IN CELL NUMBER) OF THREE CLASSES OF STAMENS (SEE TEXT) AFTER 10-DAY CULTURING ON FIVE DIFFERENT MEDIA.

Medium no.	*Class of stamens**	*Cell number/hais (SD)* *Before culture*	*After culture*	*Growth*
R1	I	7.9 ± 1.1	12.6 ± 1.8	4.6 ± 1.1
	II	12.6 ± 1.5	17.6 ± 1.6	5.0 ± 1.5
	III	15.9 ± 0.2	19.4 ± 0.8	3.6 ± 0.6
R3	I	7.9 ± 1.1	9.9 ± 3.0	2.0 ± 1.1
	II	12.5 ± 1.3	16.3 ± 2.5	3.8 ± 1.7
	III	17.4 ± 1.4	19.3 ± 1.4	1.9 ± 0.8
R5	I	8.2 ± 1.2	14.0 ± 1.6	5.8 ± 1.5
	II	12.0 ± 1.1	16.0 ± 0.8	4.0 ± 0.9
	III	17.9 ± 0.9	20.2 ± 0.5	2.3 ± 0.4
M1	I	8.3 ± 1.4	13.6 ± 1.5	5.2 ± 2.6
	II	12.2 ± 1.5	14.7 ± 4.2	3.6 ± 2.0
	III	18.3 ± 1.1	19.6 ± 1.4	1.3 ± 1.2
0	I	8.5 ± 1.2	11.0 ± 1.5	2.5 ± 2.1
	II	11.6 ± 1.3	13.6 ± 2.9	2.0 ± 1.6
	III	——	——	——

* See text.

TABLE VI

FREQUENCY OF COLORATION OCCURRED IN STAMEN HAIRS AFTER 10-DAY CULTURING ON FIVE DIFFERENT MEDIA.

Medium no.	*Number of colored stamen hairs per flower** *Class I*	*Class II*	*Class III*
R1	0.5	9.6	23.5
R3	3.6	13.7	9.9
R5	7.6	14.2	24.0
M1	2.6	21.8	80.7
0	0	0	—

* "Flower" means a cluster of five stamens plus ovary cultured. All the five stamens did not always survive.

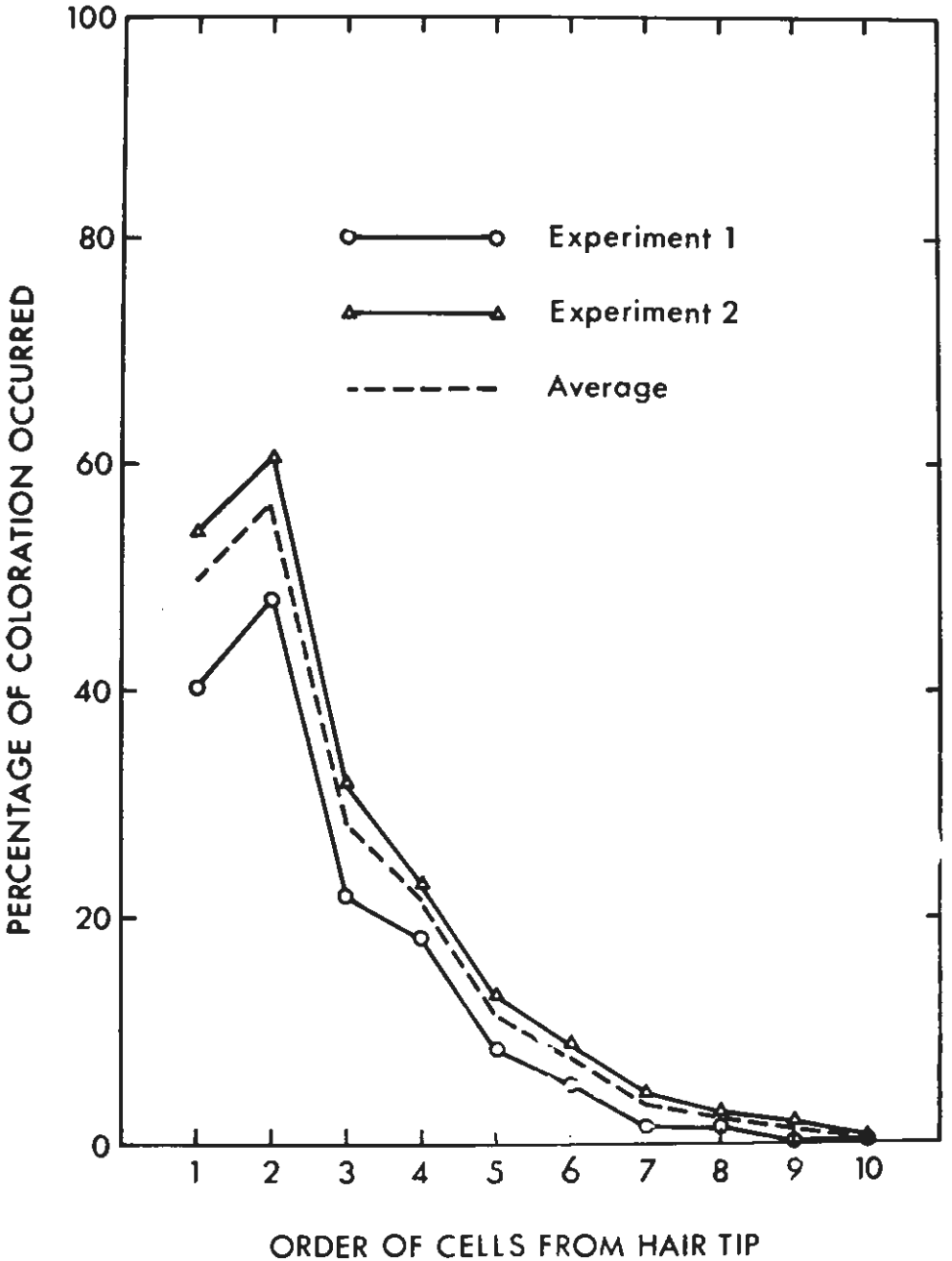

Fig. 1. Distribution patterns of colored cells in the terminal ten cells of the stamen hairs after culturing for 10 days. Pooled data from Experiment 1 (on the media 1, 2 and 3) and those from Experiment 2 (on the media R1, R3, R5 and M1) are compared.

The distributions of colored cells in the hairs of the stamens cultured on media R1, R3, R5 and M1 are presented in Table VII, and the distribution pattern is drawn in Fig. 1 together with that observed in Experiment 1. The results are similar to or parallel with those in Experiment 1; the subterminal cells coloring most frequently, the terminal cells next frequently, and the third to tenth cells following them in the order of cells from the hair tip.

TABLE VII

DISTRIBUTION OF COLORED CELLS IN THE STAMEN HAIRS AFTER CULTURING ON THE MEDIA R1, R3, R5 AND M1

Order of cells from hair tip	*Number of cells colored on*				
	Medium R1	*Medium R3*	*Medium R5*	*Medium M1*	*Total*
Terminal	104	131	186	358	779
Sumbterminal	107	134	227	404	872
3rd	42	76	92	247	457
4th	20	33	70	208	331
5th	9	33	25	122	189
6th	2	23	6	95	126
7th	2	10	5	47	64
8th	0	11	1	28	40
9th	2	8	1	19	30
10th	1	2	1	8	12
Total number of hairs Examined	187	258	332	663	1440

DISCUSSION

Culturing *Tradescantia stamen* hairs was first tried by Parchman (1962, 1964) using *T. paludosa,* to know hormonal effects on the stamen hair cells. He studied the hair growth using the index of the degre of cell division and cell products, and observed the maximum hair growth on the medium containing 10^{-7} M IAA and 10^{-6} M kinetin. The medium allowed, however, division of stamen hair cells only once (Parchman, 1962).

In the present study, on the other hand, increase of as many as ten cells per hair was observed (see Table II). Cell division in *Tradescantia* stamen hairs is known to occur mostly at the terminal two cells of each hair (Ichikawa and Sparrow, 1967). The 10-cell increase per hair means that ten cell divisions on the average occurred per hair during the culture on the best medium so far examined. The difference between Parchman's and the present results is though to be caused by the difference of the culture methods. The culture of bare stamens failed to let them grow in the preliminary experiment of this study. Culturing together with other floral organs (*e.g.*, ovary and receptacle) thus seems to be needed for a successful culture of stamens.

There has been no study on the coloration of cultured stamen hairs. As far as the present study is concerned, it seems possible to say that the coloration of stamen hairs is determined by hormonal combinations. Coloration of stamen hairs was most promoted on the medium 1 used in Experiment 1 and the frequency of colored hairs was highest on the medium M1 used in Experiment 2. These media contained 0.1 mg/*l* IAA, 1.0 mg/*l* GA_3 and 0.1 mg/*l* kinetin. The media 2, 3 and R5 also induced coloration (see Table I for their hormonal combinations). Considering these results, the hormonal conditions necessary for coloration are judged to be as follows so far as examined: At least 0.1 mg/*l* IAA, plus 1.0 mg/*l* GA_3 and 0.1 mg/*l* kinetin. Higher than 4% sucrose concentration seemed also necessary, considering the highest frequency of coloration ocurred on the medium M1 (see Table VI).

The coloration did not occur at random, and there were various patterns of coloration. It was observed that all hairs of a stamen were colored while all hairs of other stamens of the same bud remained colorless. Thus the coloration of stamen hairs seemed to be an event of all or none, highly probably having (a) trigger(s). Ordinarily, in the normal flowers of this clone, pigment formation in the stamen hairs is initiated five or six days before flowering (Ichikawa *et al.*, 1969) after completing the repeated divisions of the terminal cell. The completion of the division is thus thought to be a likely trigger of coloration.

Taking this fact into consideration, the pattern of coloration was analyzed. Distribution patterns of colored cells (from the terminal-cell position to the tenth-cell position from the hair tip) examined for colored hairs in Experiments 1 and 2 were similar to each other, and the results of the two experiments are compared in Fig. 1. As shown in this figure, the coloration occurred most frequently in the subterminal cells, and next frequently in the terminal cells. The rate of coloration decreased steadily with the distance from the subterminal cell. This relationship shows that coloration starts at the subterminal or terminal cells of hairs and proceeds down to the basal portion. It does not contradict with earlier observations of young stamen hair (Ichikawa, unpub.). Also, the idea that the completion of cell divisions is a trigger of the coloration does not contradict with these results, because the terminal and subterminal cells can receive the information of completing divisions most directly among hair cells due to the nature of cell increase in stamen hairs.

It is thought that some inhibiting mechanisms of coloring trigger can be at leasts partially broken by some combinations of hormones and also likely by a certain sucrose concentration.

The results of the present study are apparently showing a high possibility of developing successfully *in vitro* culture techniques of the stamens of *Tradescantia*.

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