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In vitro Modification of Sex Expression of Cucumber by Plant Growth Regulators

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Summary

Effects of seven different plant growth regulators on sex modification of cucumber were studied by using aseptic culture on the media. Apexes of monoecious type of cucumber at early embryonic stage were explanted throughout the experiments, and cultured under conditions which would have been potentially expected to be male flower. After 90 days culture, sexes of flowers formed on each node were observed either visually or microscopically.

Cytokinins, such as kinetin (K), N^6-benzyladenine (BA), 6-benzylamino-9-(2-tetrahydropyranyl)-9H-purine (SD8339) promoted pistillate organ development which lead to pistillate flower. Addition of mixture with NAA increased the female tendency.

Effect of gibberellic acid (GA_3) was found to promote male tendency, and increased tendency was found by adding it with abscisic acid (ABA) in the same concentrations.

Abscisic acid affected differently on sex modification depending on their concentrations.

Ethrel was the most effective substance for femaleness, but the effect was suppressed by the presence of ABA in the same concentration.

Introduction

Regulation of sex is very important for fruit vegetable production. That has been possible by environmental and chemical factors in intact plant of many different species. Particularly sex expression of cucumber can be easily modified as compared with other plants. Effect of environmental factors, such as photoperiodism and temperature, has been mainly reported, until recently when that of various plant growth regulators was studied in species of the family *Cucurbitaceae*. Studies with cucumber, muskmelon, pumpkin and *Luffa actangula* revealed that exogenous application of auxins, such as indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA)[8,21], ethylene and ethrel[3,4,7,12,16,18,20,28,32], ABA[25,27] and growth retardants, such as succinic acid 2,2 dimethylhydrazide (B-995)[5,13,20], bromocholine bromide[23], maleic hydrazide[24] and morphactin[19] enhances the femaleness, while GA has an opposite effect[26,35].

All of results mentioned above were obtained using intact plants. *In vitro* culture may be useful for investigating activity of plant growth regulators on the sex modification without obligatory interference from leaves or other plant organs. This was first examined by Galun *et al.* who cultured young floral buds at bisexual stage morphologically and physiologically[9,10]. By adding of IAA directly to the medium with or without the presence of GA_3, they could succeed to modify flowers of genetically male strain to female flower. A combination of 1.0 mg/*l* GA_3 and 0.3 mg/*l* IAA added to the medium increased pistillate and hermaphrodite flower formation in

cucumber[33].

In this paper studied the sex modification, effects of various plant growth regulators added singly or as mixture to apices of young cucumber seedling cultured *in vitro* were reported.

Materials and methods

Monoecious line of cucumber, *Cucumis sativus* L. cv. 'Sagami-Hanjiro' used in this experiment forms only staminate flowers up to the 15th node and pistillate flowers are seen on subsequent nodes higher than the 16th under summer condition of the central Japan.

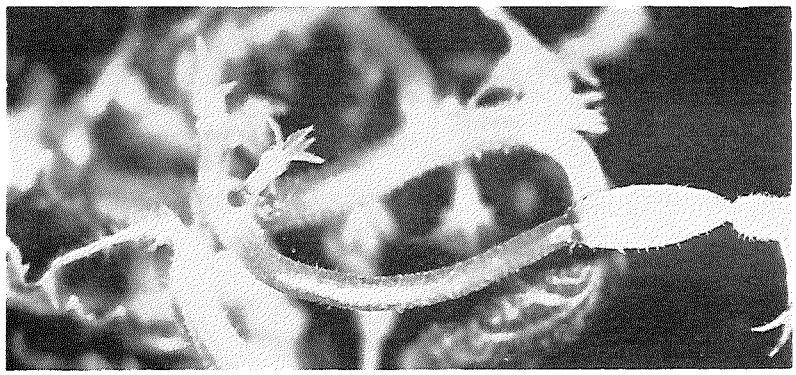
Seeds were sterilized by dipping into Antiformine solution (containing 1.0 % of active Cl), and then sown on the basal medium containing Murashige and Skoog (1962)'s inorganic salts, 3 % sucrose, 2.0 mg/*l* *myo*-inositol, 0.5 mg/*l* nicotinic acid and pyridoxine-HCl, 0.1 mg/*l* thiamine-HCl and 0.8 % agar. When first true leaf expanded after 3 weeks, shoot apex with a few leaf primordia was dissected. A dome-forming apex thus obtained was explanted to a test tube containing 20 m*l* of the medium, which was composed from the basal medium and various plant growth regulators. Naphthalene acetic acid and cytokinins were mixed with the basal medium and autoclaved. Gibberellic acid (GA_3), ethrel and ABA were first filtersterilized and then mixed with autoclaved basal medium before solidification. These substances were used at the concentrations of 10^{-2}, 10^{-1} and 1 mg/*l*. Naphthalene acetic acid was singly added to the basal medium. Cytokinin, such as K, BA or SD 8339 was also added to the basal medium with or without 10^{-2} mg/*l* NAA. Abscisic acid, ethrel or GA_3 was added singly or as mixture to the basal medium with or without 10^{-2} mg/*l* of NAA and K. All of incubation was carried out under 16 hr day-length supplemented with plant-lux lamps (Toshiba Co.) and incandescent lamps of total 3000 lux.

The apexes and young flower buds which were too small to determine sexes visually were fixed at the beginning and subsequently 3, 23, 30, 38 and 90 days by FAA to observe developmental stages. Fixed materials were dehydrated by means of the usual alcohol series, embedded in paraffin and then sectioned by a rotary microtome at 15μ. Serial sections were stained with Delafield's Haematoxylin and made to permanental histological slides, and used for the evaluation of sex expression.

Results

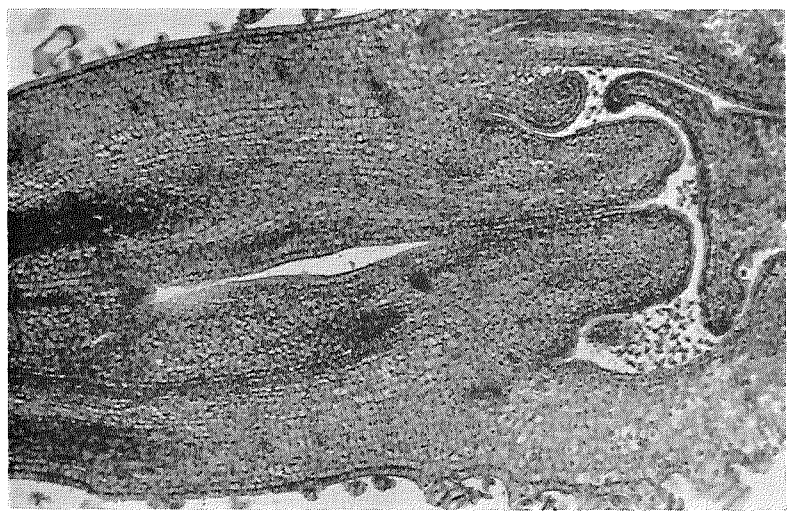
All of flower buds at the beginning of the culture was at the early embryonic stage. Twenty-three days after explanting, lateral buds initiated on more than ten nodes, and 4 to 7 leaves expanded. After 30 days, sexes of flower buds on first 3-5 nodes became possible to determine. After 90 days, sexes of most of flowers up to the 15 th node were observable. It was clear that number of staminate and pistillate flower buds differed by the media. In Tables 1, 2 and 3 were shown respectively the effects of NAA and cytokinins, NAA, K, ABA, GA_3 and ethrel, and NAA, K, ABA and ethrel, on sex expression 90 days after explanting. Number of buds younger than bisexual stages were omitted from the results. The number of staminate and pistillate flowers on each node are calculated as a total of 3 plants, plant height and number of leaves are shown as an average of single plant. Since a young shoot was cut for culture, the lst node in the tables corresponds to the 3 rd or 4 th node of an intact seedling. Pistillate flowers developed from those with bisexual organs by developing selectively only pistillate organs as shown in Figs. 1-a~c. On the other hand, pistillate

↓ Pistillate flower

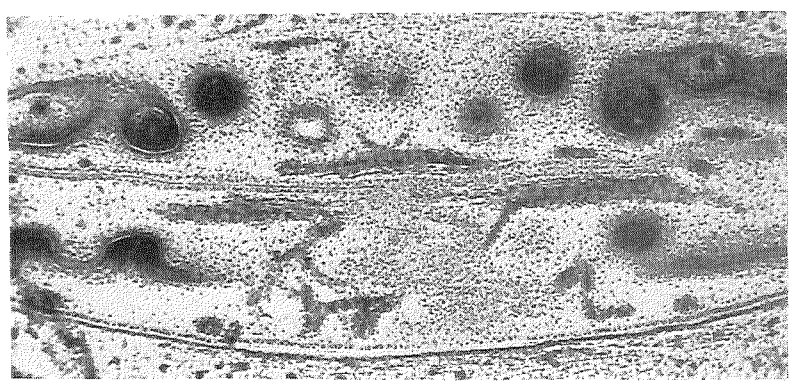


← Pistillate flower

a



b



c

Figure. 1 Pistillate flowers after 90 days of culturing in basal medium containing 10^{-2}mg/l NAA and kinetin.

a. Cluster of pistillate flowers. ×1.5
b. Longitudinal section of pistillate flower. ×50
c. Longitudinal section of ovary of pistillate flower. ×50

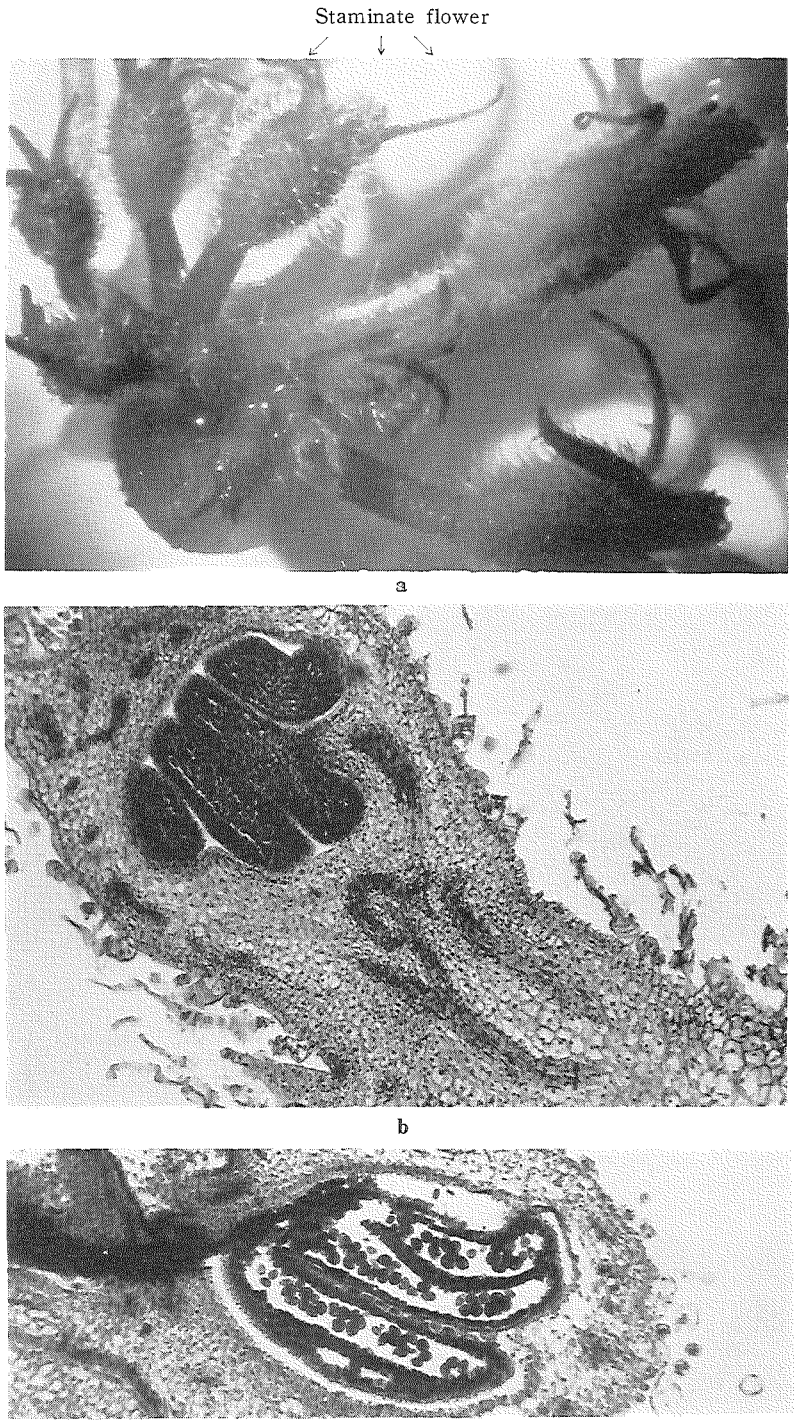


Figure. 2 Staminate flowers after 90 days of culturing in basal medium containing 1 mg/*l* ABA.

a. Cluster of staminate flowers. ×1.5

b. Longitudinal section of staminate flower. ×50

c. Pollen grains development in anthers of staminate flower. ×50

Table 1. Effects of NAA and cytokinins on a total number of staminate and pistillate flowers on each node of three plants***

Lot no.	Chemicals (mg/*l*) NAA	K	BA	SD	Plant height (per single plant)	Number of leaf	Node order on main stem 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1-1	0	0	0	0	10.2*cm*	9.3	5*	3	3	4	5	3	2	2	2						
2	10^{-2}				7.8	15.7	0	1	5	1	2 (1)**	2 (2)	1 (1)	1 (1)	1						
3		10^{-2}			9.6	9.0	0	0	0	2	6 (1)	3 (1)	(2)	(3)	(3)	(3)	(2)	(2)	(2)	(2)	(1)
4			10^{-2}		6.0	12.0	4	4	4 (1)	2	3 (2)	(6)	(2)	(1)							
5				10^{-2}	6.2	12.0	2	4	5	9 (1)	11 (2)	10									
6	10^{-2}	10^{-2}			10.7	14.0	3 (1)	1 (2)	(5)	(3)	(4)	(4)	(4)	(5)	(7)	(2)					
7	10^{-2}		10^{-2}		8.7	12.3	0 (1)	(3)	(3)	(3)	(2)	(1)									
8	10^{-2}			10^{-2}	8.8	10.3	2 (1)	(4)	(5)	(6)	(5)	(3)	(3)								

* Number of staminate flowers.
** Number of pistillate flowers.
*** Apexes of 21 days old were explanted, and then cultured for 90 days.

Table 2. Effects of NAA, K, ABA, GA_3 and ethrel on a total number of staminate and pistillate flowers on each node of three plants

Lot no.	Chemicals (mg/*l*) NAA	K	ABA	GA	Eth.	Plant height (per single plant)	Number of leaf	Node order on main stem 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1-1	0	0	0	0	0	8.9*cm*	11.7	3*	7	8	10	5	4	4 (1)**	3 (1)	6	5	3				
2	10^{-2}	10^{-2}				8.6	11.3	0 (3)	1 (4)	1 (4)		(5)	(7)	(6)	4 (1)	6 (1)	5 (2)	(2)				
3			10^{-2}			8.2	9.7	1	5	3 (1)	7 (1)	4	4 (2)	2 (2)	2	5						
4			10^{-1}			0.7	2.3	4 (1)	11	3	3 (1)		(2)	9	3	3						
5				10^{-2}		12.3	12.0	6	11	11	19 (1)	16 (1)	11 (1)	10 (1)	13 (1)	7 (2)	4 (1)	(1)				
6				10^{-1}		6.7	8.7	3	8	6	18	16	13 (1)	7 (1)	7 (1)	7	5					
7	10^{-2}	10^{-2}		10^{-2}		9.5	12.3	0	0	7 (2)	11	8	10	7	5	6	5					
8	10^{-2}	10^{-2}		10^{-1}		8.3	10.3	1	2	5 (1)	2 (1)	2 (4)	(6)	3 (3)	(4)	(3)	(4)	(1)				
9					10^{-2}	7.7	9.3	5	8 (1)	7	16	11	6 (3)	11 (1)	7 (1)	5 (1)	6					
10					10^{-1}	6.0	8.7	0	0 (2)	(5)	(6)	(5)	(9)	(3)	(2)	(1)						
11			10^{-2}	10^{-2}		13.3	11.0	10	16	14	14	14	8	11	8 (1)	6 (2)	2 (1)					
12			10^{-1}	10^{-1}		8.9	8.7	11	15	16	12	12	7	2	1	1 (1)	(1)	(1)	2 (1)			
13				10^{-2}	10^{-2}	4.4	9.7	15	13	12	10 (1)	5 (3)	3 (3)	1 (2)	(2)	(3)	(2)	(2)	(2)			
14				10^{-1}	10^{-1}	1.2	5.5	25 (3)	(3)	(2)												
15			10^{-2}		10^{-2}	2.1	4.3	31	49	16	26	11	10	9								
16			10^{-1}		10^{-1}	0.2	4.7	0														

* Number of staminate flowers.
** Number of pistillate flowers.
*** Apexes of 21 days old were explanted, and then cultured for 90 days.

Table 3. Effects of NAA, K, ABA and ethrel on a total number of staminate and pistillate flowers on each node of three plants***

Lot no.	Chemicals (mg/l)				Plant height	Number of leaf	Node order on main stem													
	NAA	K	ABA	Ethrel	(per single plant)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3-1	0	0	0	0	8.8cm	19.3	7*11		11 ①**	7	7	5	1	1							
2	10^{-2}	10^{-2}			9.5	19.7	0	0													
									①	②	③	⑤	④	②	②	①	①				
3			10^{-2}		14.4	19.3	0	0	4	3	2	3	3	3	2						
											③	③	②	②	①	①	①	①			
4			10^{-1}		17.0	12.3	0	8	16	17	15	7	6	1	5	5	5	4	2		
														①	①	①	①	①	①		
5			1		1.8	6.0	8	20	13	10	11	9	5								
6				10^{-2}	10.3	15.7	0	4	3	6	2	3	1	1	1			2	1	2	1
							①	③	③	③	②		②	②	②	③	③	③	③	③	①
7				10^{-1}	6.7	9.7	0	8	22	12	5	6	2			7	5				
									①	②	②	③	③	④	⑤	②	②				
8				1	6.0	16.7	0	0	0	0											
										③	④	⑦	⑤	④	⑤	④	④	②	②		
9	10^{-2}	10^{-2}	10^{-2}		9.2	17.0	0	2	2	2	4										
									②	②	①	⑤	④	③	③	④	③	③			
10	10^{-2}	10^{-2}	10^{-1}		8.7	13.3	1	3	7	6	4	1	1	1	1				1		
									②	①	④	④	③	①	①	①	①	①			
11	10^{-2}	10^{-2}	1		5.6	8.7	11	8	13	14	7	14	13	11	11	2	1				
12	10^{-2}	10^{-2}		10^{-2}	12.5	17.7	0	3	4	5	5	6	8	7	7	4	2	2	1		
										②	①	①	①	③	②	②	③				
13	10^{-2}	10^{-2}		10^{-1}	8.2	12.0	0														
								②	⑤	⑤	⑤	②	②	②	②	②	②	②			
14	10^{-2}	10^{-2}		1	6.2	18.0	0	0					1								
									①	①	②	②	②	①	②	②	②	②	②		
15	10^{-2}	10^{-2}	10^{-2}	10^{-2}	7.6	9.7	0				1	1	2	1		1					
								①	①	①	③	②	②	③	①						
16	10^{-2}	10^{-2}	10^{-2}	10^{-1}	8.3	15.3	0									1	1				
							②	⑥	③	⑤	④	④	②	②	②	①	①	②			
17	10^{-2}	10^{-2}	10^{-2}	1	7.3	17.3	0	0	0	3	0										
												①	③								
18	10^{-2}	10^{-2}	10^{-1}	10^{-2}	15.7	17.3	8	13	13	11	9	12	7	4	5	3	4	3	1	1	2
									①	①											
19	10^{-2}	10^{-2}	1	10^{-2}	10.9	8.3	10	20	11	9	9	7	7	2	2	1					

* Number of staminate flowers.
** Number of pistillate flowers.
*** Apexes of 35 days old were explanted and cultured for 90 days.

organ development of staminate flowers was arrested and pollen grains formation occurred in anthers (Figs. 2-a~c). Plants grown on the basal medium initiated staminate flowers on each node and a small number of pistillate flowers (Tables 1-1, 2-1 and 3-1). The 10^{-2} mg/*l* of NAA, K, BA or SD8339 increased number of pistillate flowers (Table 1-2~5). When these cytokinins and NAA were added to the basal medium, order of node which formed first pistillate flower lowered, and total number of the pistillate flowers increased (Table 1-6~8). Kinetin was found to be the most effective for the pistillate flower formation among three cytokinins tested.

Single addition of ethrel also resulted in promotion of femaleness (Tables 2-10, 3-6~8). When ethrel, NAA and K were added simultaneously to the basal medium, similar tendency was induced (Table 3-12~14). Although mixture of ethrel and GA_3 induced staminate flowers on lower nodes and pistillate flowers on higher nodes (Table 2-13), high concentration of them inhibited the plant growth and induced staminate flower cluster on a stunt shoot (Table 2-14). Mixture of ethrel and ABA increased

a number of staminate flowers (Table 2-15), and a high concentration of them arrested completely the plant growth (Table 2-16).

Single addition of ABA induced staminate flowers and a small number of pistillate flowers at lower concentration (Tables 2-3~4, 3-3~4), and only staminate flowers, at a high concentration (Table 3-5). When ABA, NAA and K were simultaneously added, pistillate flowers induced at lower concentrations (Table 3-9~10), but only staminate flowers were induced at a high concentration (Table 3-11). When ABA was added with GA_3, a number of staminate flowers on lower nodes increased (Table 2-11~12).

Single addition of GA_3 increased staminate flower formation on every node, and a small number of pistillate flowers, on higher nodes (Table 2-5~6). When GA_3, NAA and K were added, number of pistillate flowers increased at a higher concentration (Table 2-8).

A mixture of NAA, K, ABA and ethrel promoted pistillate flower formation at a low concentration (Table 3-16), but ethrel of a high concentration inhibited flower formation (Table 3-17), and ABA of high concentration promoted staminate flower formation (Table 3-18~19).

Discussion

Flower initiates as hermaphrodite one in cucumber and develops into an unisexual flower by taking any one of possible alternative pathways, which eventually lead it to either staminate or pistillate flower. It has been shown that, with previous experiments at bisexual stage, isolated cucumber floral primordia were possible to grow on the artificial medium with addition of IAA and GA_3, and possible to stimulate development of pistillate organs[9,10,33]. In this paper, effects of seven different plant growth regulators on pistillate organ development were studied, and their effects on the primordia and initiated buds under the culture conditions were observed until the 15th node. Some of substances tested were able to promote femaleness under the male promoting conditions, and some others increased or decreased the number of staminate flowers. Their effectiveness depends on their concentrations.

Cytokinins tend to promote femaleness, especially when they are added with NAA. One of cytokinins, SD8339, has been known to convert genetically staminate plants of *Vitis vinifera* to functional hermaphrodites[22]. Application of cytokinins to *Cucumis* spp. promotes fruit setting rather than sex convertion[17], and application to fig and apple promotes parthenocarpy[6,32]. These two effects of cytokinins may be expressed through stimulation and promotion of pistillate organs development. Hermaphrodite flowers were developed in many cucumber culture[9,10,33] as well as in male grapes[17]. However, in this experiment, among pistillate and staminate organs of pistillate flowers only pistillate organ was selectively developed, and this may be due to using young undifferentiated embryonic tissue from which most of buds initiated after explanting on the medium which contained plant growth regulators.

Simple application of ethrel showed an tendency to femaleness by the fact that nodes bearing many pistillate flowers per each node continues. Actually, this chemical is the most effective one on promoting femaleness in intact androecious and monoecious cucumber[3,4,7,16,20,28,31]. Presumable exogenous plant growth regulators which are applied at bisexual stage or much younger stage could directly or indirectly change levels of endogenous plant hormones, and in turn stimulate their development to either staminate or pistillate organ. That this may be true can be deduced from two reports which

indicate the femaleness is strengthened by a high endogenous level of auxin or ethylene. The first one is that apices and young shoots of hermaphrodite plants of cucumber contained high amount of auxin than those of andromonoecious one[11], and the second, endogenous ethylene level in apices is higher in gynoecious cucumber than is in monoecious one[30].

There are many reports which indicate the application of exogenous plant growth regulators to a plant could change amounts of endogenous hormones in a treated plant. Since auxin treatment induced ethylene evolution[1] and also ethephon promoted evolution of endogenous ABA[31], ethylene could be more directly for induction of femaleness than does auxin, and ABA might be more direct regulator than ethylene[31]. In spite of that, in this experiment, ABA affected promotion of maleness rather than femaleness, and same concentration of ABA suppressed the effect of ethrel. Mixture addition with GA_3 also promoted maleness. The difference of ABA activity in the present study on sex expression is considered to be due to the difference that plantlets *in vitro* were used.

Gibberellic acids are known to modify sex expression of genetically female cucumber strain under ordinary condition, therefore it may increase staminate flower formation and at the same time delays pistillate flower formation[26,27,29]. In female plant of *Cannabis sativus*, GA induces the staminate flowers, and application of mixture of ABA abd GA_3 inhibits this male tendency by GA_3[25]. Since endogenous level of GA-like substance in a monoecious cucumber was higher than in gynoecious one[2,14,15] and ethephon also reduce GA activity[31], the results are cleary indicating that, at least in these species a high endogenous level af GA stimulates maleness. With *in vitro* experiments it has been reported that GA does not affect on sex conversion[9,10] and has some roles to feminize floral buds[33]. In this experiment, GA_3 increased a number of staminate flowers beared per node. Moreover, mixture application of GA_3 and ABA increased number of staminate flowers, and with the presence of ethrel, GA_3 suppressed the female promoting effect of ethrel. These results with detached plantlets clearly indicate that GA_3 can promote male tendency, as has been known previous experiments with intact plants.

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人工培地上でのキウリ花芽の植物生長調節物質による性転換

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人工培地上で培養したキウリ花芽の，性表現を転換する効果を，7種類の植物生長調節物質を用いて研究した．雌雄同株型のキウリの，播種後3週間位の若い生長点を切り出し，人工培地上に植付けた．この人工培地には，ムラシゲ・スクーグの基本培地に，NAA，3種のサイトカイニン（カイネチン，ベンジルアデニン，SD8339），ジベレリン酸（GA_3），アブサイシン酸およびエスレルを種々の濃度，種々の組合せて添加した．これらの培養物は，25°C，16時間日長下で90日間培養後，各節毎の花の性を観察した．この環境条件は，キウリのこの品種では雄花となりやすい条件である．

3種のサイトカイニン類は，花芽の子房肥大を促進して雌花とする効果があり，NAA と加用されるとその効果がいちじるしかった．

GA_3 は，雄花数を増加する効果がみられ，同濃度のアブサイシン酸と加用すると，その効果が促進された．

アブサイシン酸は，濃度によって性転換におよぼす効果が異った．

エスレルは，雌花化促進に最も効果があったが，同濃度のアブサイシン酸加用は，その効果を打消した．