Studies on the photoperiodic sex differentiation in cucumber, Cucumis sativus L. : I. Effect of temperature and photoperiod upon the sex differentiation

Matsuo, Eisuke
Horticultural Laboratory, Department of Agriculture, Kyushu University

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Studies on the photoperiodic sex differentiation in cucumber, *Cucumis sativus* L.

I. Effect of temperature and photoperiod upon the sex differentiation

Eisuke MATSUO

Since Garner and Allard (1920) reported the photoperiodism in the flowering of tobacco plant, a number of workers confirmed the three basic types of plants of photoperiodic responses ; long-day plant, short-day plant and day-neutral plant. Moreover, the photoperiodism has been found universally with growing and developmental responses in plants, not only flowering but also tuberization, bulb formation, resting bud formation in trees, breaking of dormancy, formation of antheridia and archegonia in mosses, etc.

On the sexuality of higher plants, since Schaffner (1923) reported that sex expression in hemp had been changed by daylegth, such a changing of sexuality by daylength has become to be confirmed in various hemp forms (McPhee, 1924, Schaffner, 1931), maize (Schaffner, 1927, Rickey and Sprange, 1932, Galinat and Naylor, 1951), cocklebur (Neidle, 1938), ragweed (Jones, 1947, Thomas, 1956), spinach (Janick and Stevenson, 1955), ramie (Shibuya, 1937), mulberry (Minamizawa, 1963), squash (Nitsch et al., 1952), muskmelon (Brantley and Warren, 1960), etc.

With regard to the sex expression of cucumber, *Cucumis sativus* L. ($2n=14$) (Heimlich, 1927), since Tiedjens (1928) reported the change of sex expression with various daylengths, this result was reconfirmed by a number of workers. It8 *et al.* (1938) found that the degree of bearing female flowers and sensitivity for the female flower formation to environmental factors were different hereditarily from variety to variety. It is well known in cucumber, however, that, the degree of female flowers and the responses of female differentiation to external stimuli are not fixed but changeable, and the sex expression can be changed by the environmental factors such as temperature, daylength, moisture condition, etc., and artificial means such as transplanting (Kurashige, 1931), pinching of flowers, leaves or of shoots (Asami *et al., 1936,* Itô and Saitô, 1957), watering (Fujii *et al., 1954,* It8 *et al.,* 1958), nitrogen fertilizing (Tiedjens, 1928, It8 *et* al., 1958, 1960) and gassing (Minina and Tylkina, 1947).

Lately, the attention has been paid to the chemical control of sexuality in cucumbers with certain growth substances, such as IAA, NAA, MH, GA, etc. (Laibach and Kribben, 1949, Itô and Saitô, 1956a, b, Galun, 1956, Wittver and Bukovac, 1958, Peterson and Anhder, 1960, Galun *et al.*, 1962, Fujieda, 1963, Saitô and Itô, 1963).

Many inquiries have been done on the temperature and daylength among the natural environmental conditions by which sex differentiation of cucumbers is affected (Tiedjens, 1928, Edmond, 1930, Itô, 1935, Itô *et al.*, 1938, Nitsch *et al.*, 1952, Shifriss and Galun, 1956, It8 and Saitô, 1956, 1957a, b, c, d, 1960, 1962, Sekiya, 1957a, b, Galun, 1961, Fujieda, 1965, 1966). As the results, it is well known that generally in cucumbers SD and/or low temperature promote female flower formation.

In spite of numbers of works on the daylength and temperature conditions for sex differentiation, the photoperiodism in the sex differentiation of cucumbers has not been studied so analytically as those in flowering, tuberization and in dormancy.

The present author discovered an interesting variety which promotes female flower formation under long-day conditions, and using this variety, he has studied the photoperiodic responses in the sex differentiation of cucumbers comparing with the basic characteristics of those in flowering and in tuberization.

The series of detailed researches on this subject will contribute to an understanding of the problems not only of sex differentiation but also of the photoperiodism itself, of the connection between flower formation and sex differentiation, of the evolution of sex pattern in plants, and also of the adaptation and formation of ecotypes. Moreover, these investigations will also contribute to the agricultural production through the chemical control of the sexuality in plants.

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Materials and methods

All the seeds used in the present experiments were supplied from Kurume Branch of the Horticultural Research Station, Ministry of Agriculture and Forestry, Japan, having high genetical purity.

In Experiment 1, several representative varieties of each of different ecotypes were used. In Experiments 2-4, variety "Higan-f ushinari" was mainly used and variety " Sagami-hanjiro " was used supplementarily. Variety "Higan-f ushinari" is the only one variety that bears male flowers under short-day condition and female flowers under long-day condition (Fukushima and Matsuo, 1965). It belongs to a variety-complex of hybrid nature. It was selected out of the offsprings of "Natsu-fushinari" X"F_1 (Natsu-fushinari X Kurume-ochiai No. 1)" and registered by the ministry as "Kyûri Nôrin No. 5" in 1962 (Fujieda *et al., 1965).* The observation on the sex differentiation in the fields showed that low temperatures promoted female flower formation, and it was not dependent on daylength and this variety bore many female flowers not only in spring but also in summer (Fujieda *et al.,* 1965). One of the characteristics of sex expression in the main stem is that the staminate phase is followed by the pistillate (These terminology will be described later in detail). This variety is cultured as "Haru-kyûri", spring cucumber, mostly in Kyushu, when the weather is rather cold and daylenth rather short. Usually it is grown in greenhouse while it is young. The optimal temperature for growth is rather high and it does not elongate too much even at 33 ℃ (Fukushima and Matsuo, 1965).

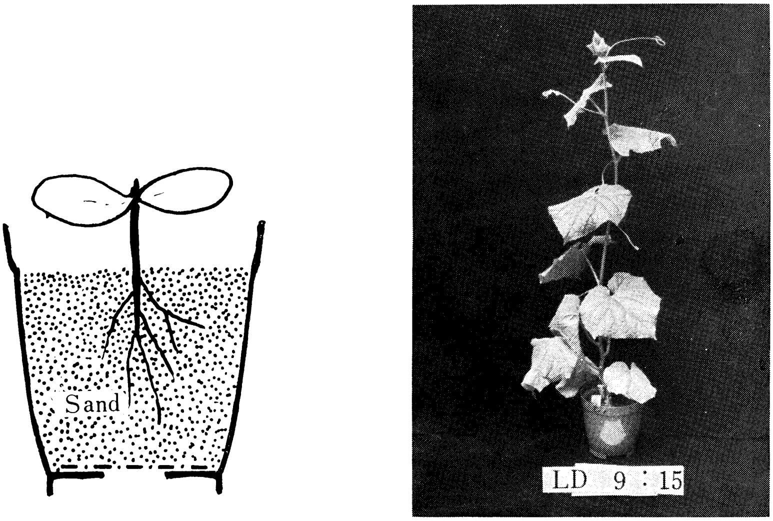

Fig. 1. Culture method and a cucumber plant.

Variety "Sagami-hanjiro" is one of the Hanjiro strain which belongs to the South Chinese type. It is well known as a variety which bears much more female flowers in low temperature and/or in short-day condition. Using this variety, Itô *et al.* have studied the sex control by daylength,

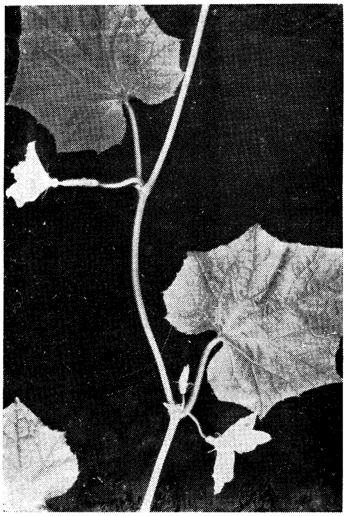

Fig. 2. Male node (lower) and female node (upper) in a cucumber plant.

temperature and application of growth substances. In general, it is known that the staminate phase in this variety is followed by the pistillate phase. In the author's experiments, however, the monoecious phase was observed after bearing the first female node. This difference in sex expression may depend on the difference of strains and experimental conditions. This variety is cultured as "Haru-kyûri," spring cucumber as same as " Higan-fushinari," mostly in Kantô district.

Seedlings, germinated in darkness at 30-33 °C, were transplanted and grown in 10-X 10-cm tin cans for Exp. 1 and in plastic pots (Fig. 1) for Exps. 2-4, which is 10.5 cm in diameter and 10 cm in depth, filled with sand, and watered with the Hyponex 1000 times solution.

Experiment were carried out in the Phytotron attached to Faculty of Agriculture, Kyushu University (In April, 1966, this Phytotron became one of the facilities of Biotron Institute, Kyushu University).

The relative humidity in the Phytotron was maintained at 60-70 %, and the average temperature was kept within the deviation of ±0.5 °C. Number of plants used in Exp. 1 was 4-6 and was 20 at greatest, 5 at least, and 8 on an average in Exps. 2-4, for it is known that plants in controlled environments grow with quite favourable uniformity and the high accuracy of the experiment can be obtained with rather a few experimental materials (Went, 1950).

Photoperiodic and temperature treatments were begun soon after the transplanting and were done throughout the experiments. Plants received daily 9 hours of natural light from 8 : 00 A.M. to 5 : 00 P.M. Those put under longer photoperiods received fluorescent light at intensities of about 500 lux at plant height for the required number of hours beginning at 5 : 00 P.M. The detailed methods will be described later with each experiment.

To denote the effect of environmental conditions on the sex differentiation in ***Cucurbitaceae,*** the sex ratio, that is, number of male flowers to that of female flowers had been used previously by many workers (Tiedjens, 1928, Edmond, 1930, Whitaker, 1931, Brantley and Warren, 1960, Hopp, 1962, ***et al.).*** It was, however, very inconvenient and was not thought to be a good denotation, because, 1) while it is obtained by counting numerous flowers on each plant, it is a quite time-consuming procedure, 2) as ***Cucurbitaceae*** has an indefinite inflorescence, the sex ratio is changeable with numbers of nodes counted, 3) therefore, it is very difficult to know how many nodes are to be counted to denote the effect of short durations of treatment upon the sex differentiation, 4) it is not fixed with varieties. Nowadays, therefore, to denote responses to treatments, the first female node, the lowest definite node on the main stem, phase, number of female nodes until a certain node on the main stem, relationship between percentage of female node and node location (See Fig. 3) are usually used according to Nitsch et ***al.*** (1952), Galun (1956, 1961), Schifriss (1961) and others. In the present paper, the above notation was used. The observation of sex expression was done when the flower organs were observed with naked eyes.

Terminology and abbreviations used in this paper are shown in Figs. 2, 3

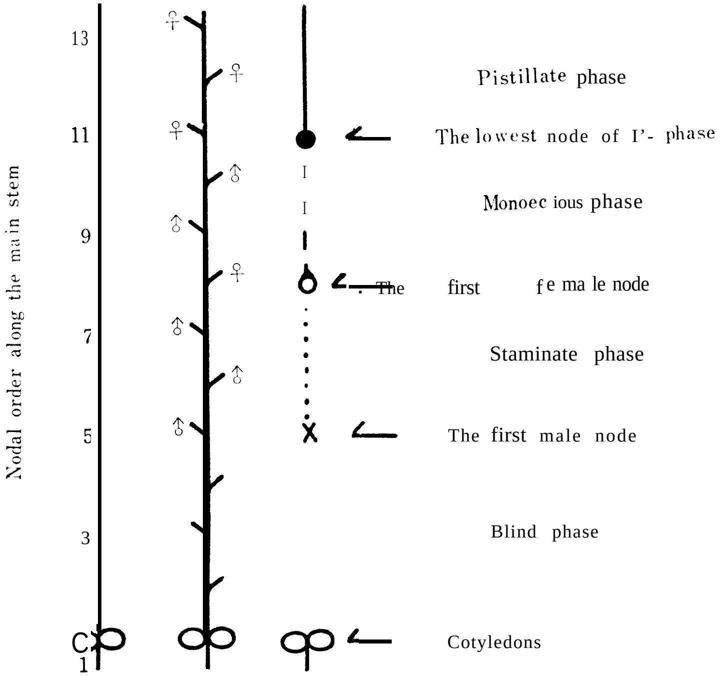

Fig. 3. The scheme of sex expression of cucumber seedlings.
P-phase : Region of female nodes only.
M-phase : Region of mixed male and female nodes.
S-phase : Region of male nodes only.
B-hase : Region of no flowers developed.

and as mentioned below ;

Male node : Node which bears male flowers.

Female node : Node which bears female flowers.

Blind node : Node which bears underdeveloped flowers or on which no flowers were observed.

Pistillate phase or P-phase : Region of the main stem bearing exclusively female nodes.

Monoecious phase or M-phase : Region of mixed male and female nodes.

Staminate phase or S-phase : Region of male nodes only.

Blind phase or B-phase : Region of no flower or underdeveloped flower nodes.

LD : Long day, SD : Short day, LDP : Long day plant,

SDP : Short day plant, DNP : Day-neutral plant.

LD 9 : 15 : Reference to light-dark cycles will be made by using symbols such as LD 9 : 15. This refers to a light-dark cycle with light for 9 hours alternating with darkness for 15 hours. The period of the cycle is the sum of the two times, namely 24 hours in this case (Hastings, 1964).

Results

Experiment I. Responses of sex differentiation to photoperiod and temperature

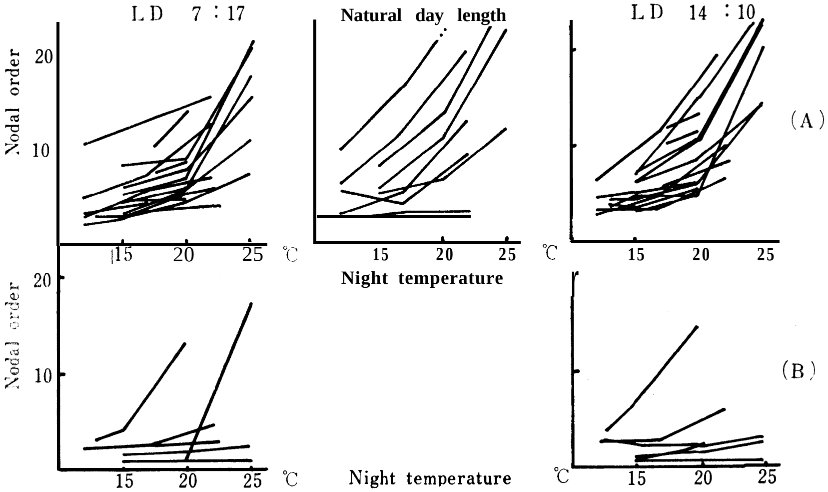

Fig. 4. Effect of temperature on the sex differentiation in cucumbers. (A) : Location of the first female node. (B) : Location of the lowest node of P-phase.

Plants received 7 hours of natural light through glass. Additional illumination, when necessary, was given as the artificial light from fluorescent tubes giving approximately 600 lux at plant height. During the 7 hours of natural day light and its before and after 30 minutes, plants were subjected to a given temperature referred to as "day temperature," and the temperature during the remaining 16 hours are called "night temperature"

Table 1. Cucumbers used for Exp. 1.

Ecotype	Cultivar
South Chinese type	Sagami-hanjiro, Kurume-ochiai No. 1, Kurume-ochiai No. 2, Hyuga No. 2, Jibai-ao, Daisen-fusinari No.1, Ao-fushinari
Hybrid strains	Higan-fushinari, Natsu-fushinari, Shogoin, Daisen, Daisen-kema, Izumi-haru, Chikanari-santo, Chojitsu-ochiai
North Chinese type	Suyo, Santo, Peking-suyo
European type	Improved Long 'Green (Netherland), Baby (USA), MSU 713-5(USA), Delikatess (Germany), Le Généreux (France), Calakhovskij (USSR), Nezhinskij mestnyi (USSR), Klinskij mestnyj (USSR)

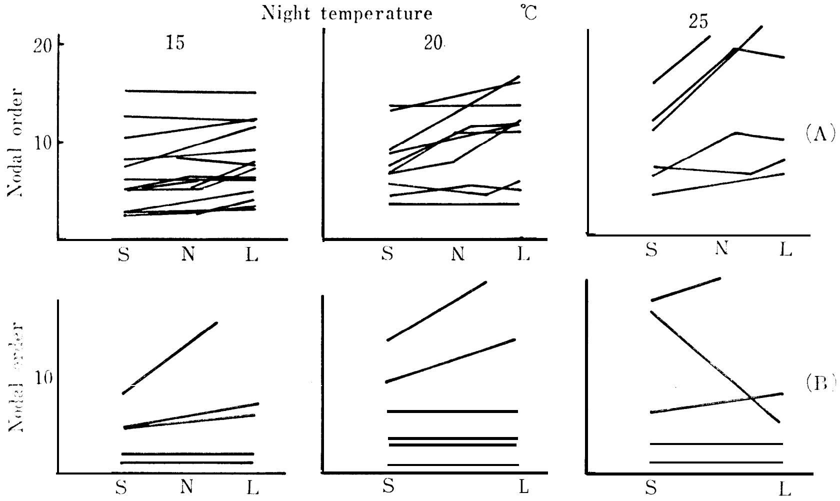

Fig. 5. Effect of photoperiod on the sex differentiation in cucumbers. (A) : Location of the first female node. (B) : Location of the lowest node of P-phase. S : Shortday, L : Longday, N : Natural day length.

regardless of whether or not the additional light was given during this period. Day temperature was 5 °C higher than the night temperature. The temperature was changed automatically. The first female node and the lowest node of pistillate phase were observed to denote the response of sex differentiation to temperature and daylength. Those results were summarized in Figs. 4 and 5. Cucumber varieties used in this experiment were listed in Table 1.

The author did not observe bisexual flowers, though Itô *et al.* (1953) observed a few ones. It was confirmed that low temperatures in any photoperiods promote female flower formation as it has been well known. In a few varieties, however, response of sex differentiation was insensitive to temperature, that is, female flower formation was not affected by temperature (Fig. 4). Concerning photoperiodic conditions, in **low** temperature conditions no differences were observed in the first female node and the lowest node of pistillate phase between the two photoperiodic conditions. In higher temperature, the first female node and the lowest node of the P-phase were higher in LD conditions than in SD ones. Only with one variety among the used ones, it was found that LD rather than SD promoted its female flower formation in higher temperature (Fig. 5).

Experimeut 2. *Minimum length of nycto-period reqnired for male flower differentiation*

The author discovered a variety which bore much more female flowers in LD condition than SD one at the temperature of 30°C in day and 25°C at night (Exp. 1). This experiment using this variety "Higan-fushinari" was performed to know the critical dark period required for the male differentiation, in other words, the critical daylength for the female differentiation.

Seedlings were grown at the temperature of 30 °C or 33 °C throughout the experiment. Plants received 8 or 9 hours of natural daily light. As in Exp. 1, additional illumination, when necessary, was given as artificial light from white fluorescent tube giving approximately 200 lux at plant height.

The results summarized in Fig. 6 show that the critical dark period for the male differentiation

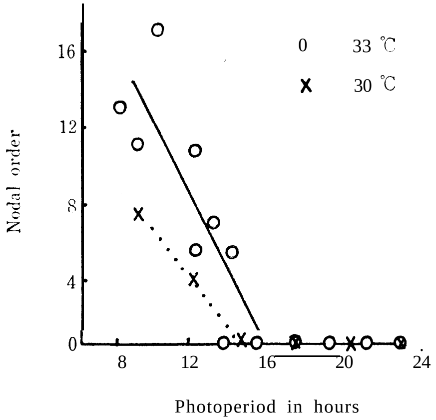

Fig. 6. Relationship between photoperiod and the highest male node of M-phase.

was about 9 hours at 30 °C. This means that this variety did not differentiate any male flowers in the photoperiods of more than 15 hours at 30-33 °C.

Experiment 3. *Effect of constant temperature*

In Exp. 2, the author confirmed the critical dark period for the male differentiation was 9 hours. As the next item of the experiment, the effect

Table 2. Sex expression of cucumbers grown under the constant high temperature of 33°C (Exp. Z-l).

Cultivar	Daylength	Average nodal position of the first female node	Average location of the lowest P-phase node
Sagami-	c	20.8	
hanjiro	N	21.8	23.8
Higan-	C	7.7	17.1
fushinari	N	3.0	22.4

N. B. C : Continuous light (Plants received 7 hours of sunlight and 17 hours of supplemental light from fluorescent lamps).
N : Natural day length (May-June).

Table 3. Sex expression of cucumbers (Exp. 2-2).

Cultivar	LD	Temp. °C	Location of the node First male	First female	Lowest P-phase	Number of female nodes until the 20th node	Frequency of gynoecious plants
Sagami-hanjiro	Natural	33	3.2	21.8	27.2		
		30	1.8	21.8	24.6		
		25	2.0	14.8	21.5		
		20	2.0	10.2	19.0		
Higan-fushinari	8:16	30	4.0	2.0	18.8	8.3	0/8
		25	3.2	2.0	6.7	15.9	0/8
		20	–	2.0	2.6	18.5	5/8
	Natural	30	7.2	3.2	20.4	8.4	0/8
		25	4.0	2.2	10.0	13.8	0/8
		20	–	1.8	2.0	19.0	8/8
	24:0	30	–	12.0	12.0	7.7	8/8
		25	–	4.0	4.0	16.4	7/8
		20	5.4	2.8	2.8	17.3	6/8

of constant temperature on sex differentiation in **LD 9 : 15** and continuous light was examined comparing with "Higan-fushinari" and "Sagami-hanjiro."

As same as observed in Exp. **1,** photoperiods counterworked on the female differentiation of these two varieties. That is, female differentiation was promoted by LD condition in "Higan-fushinari" and by **SD** condition in " Sagami-hanjiro " (Tables 2, 3 and 4). In both varieties, however, low temperature promoted female differentiation. For " Higan-fushinari " promotion of female differentiation by low temperature was more remarkable in SD than in LD condition. In continuous light male differentiation was observed at lower temperature rather than higher temperature, and female plants which had no male flowers was more frequent at higher temperature than lower one (Tables 3 and 4).

Table 4. Sex expression of cucumber "Higan-fushinari" (Exp. 2-3).

LD	Temp. °C	Location of the node First male	Location of the node First female	Location of the node Lowest P-phase	Number of female nodes until the 20th node	Frequency of gynoecious plants
24 : 0	30	5.0	3.0	3.8	17.7	5/6
	25	6.4	3.0	5.3	17.3	7/12
	20	4.3	2.2	4.3	18.2	6/12
9 : 15	30	6.8	3.0	12.2	13.2	0/6
	25	3.7	2.7	9.0	14.3	0/12
	20	2.9	2.0	3.8	18.2	3/12

Experiment 4. *Effect of alternative temperature*

In Exp. 3, the author with " Higan-fushinari" confirmed that the lower the temperature was, the more noticeable the female differentiation was in SD, and that in continuous light a few male flowers differentiated at lower temperatures. Following experiment was performed to know the effect of alternative temperature not only the sex expression but also on the growth of cucumber seedlings.

" Higan-f ushinari " seedlings received 9 hours of natural daily light from 8 : 00 A.M. to 5 : 00 P.M. Temperature was changed twice automatically at 9 : 00 A.M. and 5 : 00 P.M. That from 9 : 00 A.M. to 5 : 00 P.M. was called phototemperature and the rest one was called nyctotemperature regardless of whether it was dark or not. Photoperiodic and temperature treatments were given at the age of the first true leaf unfolded. After the treatment of 30 days, sex expression was observed. As plant growth was inhibited and sex of flowers was not ascertained at 20 ℃ of phototemperature, treatment was prolonged for more than two weeks until the sex of flowers could be distinguished.

*Growth **of seedling:***

The growth of seedlings was expressed with the number of unfolded true leaves and stem length, and was shown in Table 5. Both in longer and in shorter photoperids, growth of seedlings as shown in number of unfolded leaves and stem length was greater at higher phototemperature than at lower one. Number of unfolded leaves increased with 25 °C nyctotemperature and 32 °C phototemperature. At 20 °C phototemperature, however, it was greatest at 25 °C nyctotemperature and least at 20 °C. Stem elongation was greatest at the nyctotemperature of 25 °C regardless of phototemperature. These results will suggest that the growth of " Higan-fushinari " is bound to phototemperature, that is, if the phototemperature is favourable, around 5 °C lower nyctotemperature than phototemperature will bring about the best growth and that when the phototemperature is unfavourable, the same or high nyctotemperature con-

Table 5. Effect of temperature on the growth of cucumber seedlings.

Phototemperature °C		32			25			20		
Nyctotemperature °C		32	25	20	32	25	20	32	25	20
Average number of unfolded leaves	LD 9 : 15	8.9	8.5	6.4	7.0	6.6	5.0	5.0	5.3	3.8
	LD 24 : 0	9.0	8.8	7.4	7.5	7.3	6.1	5.5	5.4	4.6
Average stem length (cm)	LD 9 : 15	35.5	47.4	26.6	15.8	26.6	19.0	7.0	11.2	6.6
	LD 24 : 0	19.6	31.0	26.8	14.3	21.6	20.1	7.6	10.3	6.9

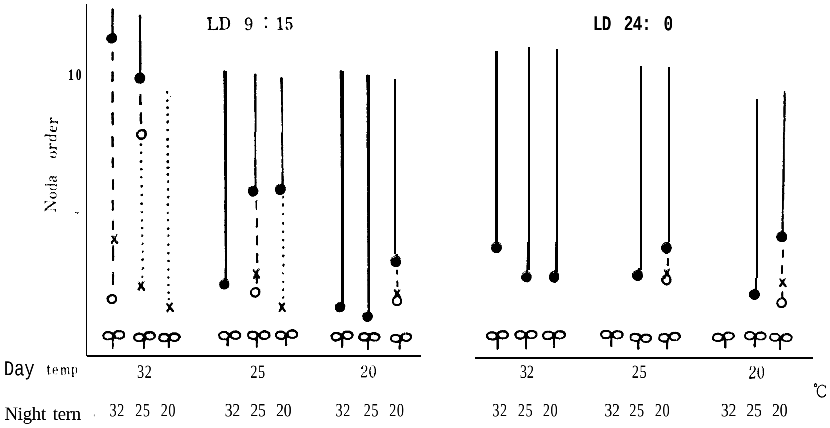

Fig. 7. Effect of temperatures on the sex expression in cucumber "Higan-fushinari."

dition is better for growth. It is well known that low nyctotemperature is favourable for plant growth (Went, 1957). The author's results, however, show that low night temperature is not always necessary for plant growth and that necessity of low night temperature depends on the day temperature. It may be necessary to study much more the optimal temperature for growth and the amount of temperature difference between day and night.

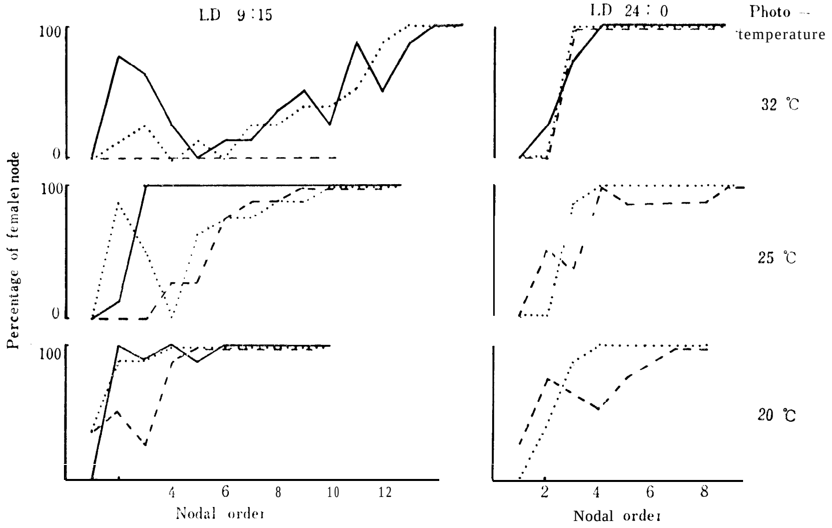

Fig. 8. Effect of temperatures on the sex expression in cucumber **"Higan-fushinari."** Nyctotemperature : 32°C (——), 25°C (······) and 20°C (----).

Sex *expression :*

Results were summarized in Figs. 7 and 8. Continuous light inhibited the development of flower organ. This tendency was greatest in the condition of 32 °C nyctotemperature and 25 °C or 20 °C phototemperature, and sex of flowers was not noticeable while the flower buds were present. In constant temperature condition, SD lowered the first female node and increased female nodes. A few male nodes were brought about at 20 °C nyctotemperature in LD conditions. The influences of temperature was greater in SD rather than LD. The lower phototemperature brought about the more female nodes and there was a tendency that low nyctotemperature increased male nodes in SD and LD.

Discussion

It is well known that the sex expression or sex pattern is changed by environmental conditions, though it is definite hereditarily. In general, cucumbers bear monosexual flowers on the axils, and their sexuality is controlled by the environmental conditions. Cucumbers bear male flowers on the proximal part of the main stem, while plants are young, and female flowers on the distal part of the main stem when they get older. This tendency is found in all of ***Cucurbitaceae*** (Nitsch et al., 1952, Brantley and Warren, 1960). Nitsch et al. studied in detail the temperature and photoperiodic conditions which are effective on the sex expression and these conditions as shown in Fig. 9. They concluded that there is a gradual shifting from the production of male to the production of female flowers under uniform environmental conditions. During this transformation, the plant passes through a succession of phases, the order of which is invariable. The climatic factors modify the length, but not the order of each phase. In the flowering sequence of cucumber, Galun (1959) summarized that as there exist three phases, i. e., male, mixed and female, any treatment or any genetic character, which brings the mixed and the female phase closer to the base of the plant, causes a more female sex exression, while any treatment, which prolongs the duration of the first phase, delays the appearance of the second phase or suppressing it altogether, resulting in male sex exression. On the other hand, Schaffner (1921) stated that the sexuality of flowers has no relation to a reduction or segregation of chromosomes or their possible hereditary factors, but that it belongs in the domain of physiological state of protoplasm and it is apparantly conditioned and determined by physiological gradient of cells. That is, flowers are sexually dimorphic originally and sex determination

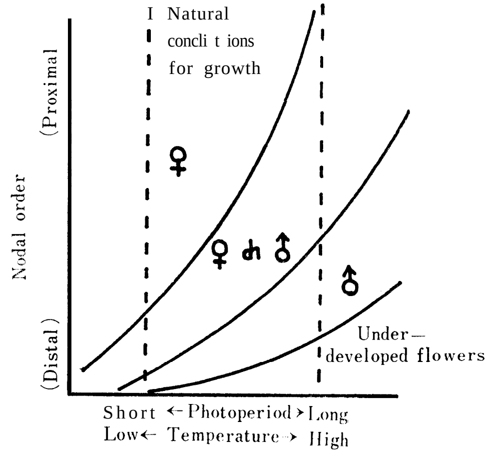

Fig. 9. Schematic representation of the effect of photoperiod and temperature on the sex expression of cucurbits (Modified data from Nitsch ***et al, 1952).***

of flowers is brought about through differential states with environmental factors. Robins and Jones (1925) with ***Asparagus*** showed that flowers are hermaphroditic primarily.

The cucumber is monoecious and shows the tendency of gradual feminization with age. In discussing the sex differentiation of cucumbers, therefore, the sex differentiation of the individual flowers on an axil and the changing of sex expression of a plant should not be confused.

Judson (1929), Fujii and Takahashi (1953) seem to have thought that male and female flowers were decided hereditarily. According to the detailed anatomical studies by Judson (1929), Fujii and Takahashi (1953), It8 et ***al.*** (1954) and Fujieda (1966), it is observed, however, that both male and female flowers originate from the bisexual situation at the very beginning of the flower development. Laibach and Kribben (1949), Itô et ***al.*** (1956a, b, 1957), Galun et ***al.*** ***(1959,*** 1962), Saitô and Itô (1963), Fujieda (1963) and others, applying growth substances to developing flowers, observed that the flowers which is expected to be male became female, and vice ***versa.***

These results support the thought that the flower itself is hermaphroditic at the early stage of its development, and at the following stage of development, it is directed to male, female or rarely hermaphroditic one by environmental conditions. The sex determination of each flower may be dependent on the certain balance of florigen-like substances and/or of the unknowns.

From the followings it is clear that there is a time lag between the flower initiation, following development of flower and the sex differentiation, and this means that sex of flowers is not yet fixed during the initial stage of flower formation. (1) It is possible to change the sex of flowers with growth substances (Laibach and Kribben, Galun ***et al.).*** ***(2)*** Appearance of the blind nodes as shown in Exp. 4. (3) Inhibition of flower development by continuous illumination (Fujieda, 1965, the author's unpublished data), and by GA treatment (unpublished data) and by red light (unpublished data). These situation will suggest that the photoperiodism will affect the sex differentiation and the development of flowers in cucumber, thinking of the Eguchi's claim (1937) that as there are two different stages in the course of flower formation, i.e., the differentiation stage of bud and the subsequent stage of development into flowering, so that photoperiodism must be discussed not only in flower initiation but also in the subsequent development of flowering.

Gradual feminization with age in cucumber may be compared with the flowering behaviour that many plants are inclined to get flowers, when they grow up. It may be thought that in flowering there exist two different reaction systems, one induces flower initiation and the other has the antagonistic action against the former, retaining vegetative growth, and that the number of flowers increases with age relatively to the latter and flowering takes place (Esashi, 19614966). In sex differentiation, therefore, it may be possible to think that there are two reaction systems, one leads flowers to female and the other has the antagonistic action against the former reaction and induces male flowers, and that the former reaction becomes to increase relative to the plant age, and in consequence, the gradual feminization will take place along the main stem of cucumber.

In flowering, younger plants have low sensitivity to the environmental factors. This sensitivity increases with plant age. In general, plants have a tendency to make the transition to the reproductive stage without difficulty as they age. Concerning this it is well known that there are three types of daylength requirement. Plants belonging to the first type show a qualitative requirement of daylength, never losing the daylength requirement though it decreases, and continue their daylength requirement infinitely so long as ordinary temperatures are supplied (begonia and cocklebur). Plants of the second type are those having a quantitative requirement, losing their daylength requirement with age, and begin reproduction (soy bean, rye and wheat). Plants of the third type are called day-neutral plants which have no photoperiodic requirement for their reproduction from a relatively early period after germination (tomato and sunflower) (Esashi, 1963).

Daylength requirement for sex differentiation in cucumbers may be compared with that for flowering. Most of the cucumbers have a tendency to make the feminization in flowers. The hypothesis in sex differentiation that the sexuality of flowers is determined by the two reaction systems, one leading flowers to female and the other to male, will give the following explanation to the gradual feminization in a plant as stated by Nitsch *et **al.*** (1952) with cucurbits and by Galun (1959) with cucumber : When plants are young, the reaction system which leads flowers to male occurs prior to the other reaction system which induces female flower. When plants are getting older, however, the latter will become superior to the former. Thus, female flowers increase with aging. When the external stimuli are given to this aging process, they will change the balance of the two reaction systems and can modify the length, but not the order of each phase. Sometimes, however, they will induce the partial change of sex expression of a plant, or some phases will be diminished in amount so that they can not be observed. The former means that the effect of the treatment is localized to the short part of the stem (8 and 9 in Fig. 10). The latter means that the staminate phase, monoecious phase and/ or pistillate phase will disappear (2-7 in Fig. 10). Which sex expression occurs on a plant seems to depend on the duration of the treatment and the age when plants are treated.

Itô (1935) reported that a certain prosperous growth takes place with many male flowers and growth inhibition with female flowers. The same phenomena were observed on the treatments with nitrogen supply (Hall, 1949, Itô et al., 1953), daylengths (Hall, 1949), growth substances (Fujieda, 1963), shading, change of temperatures, etc. Fujieda (1963) thought that the elongation and sex differentiation are controlled simultaneously by a certain kind of endogeneous material, probably one of the growth substances, because the great elongation takes place with many male flowers and many blind nodes when plants are treated with **GA.** It is wellknown that in most of cucumber varieties including "Higan-fushinari," when their growth is inhibited, they become to have many female nodes, and also the development of flowers is advanced on the lower part of the stem. This may suggest that there is a certain relationship between the elongation and the flower development including sex differentiation. However, as there exist time lag between the flower formation, flower development and sex differentiation, it is hard to think that the elongation and male flowers (or growth inhibition and female flowers) are fixed simultaneously by a certain material. The following facts may also support this consideration. The elongation was greater at high day temperature than at low day temperature and took place male flower forma-

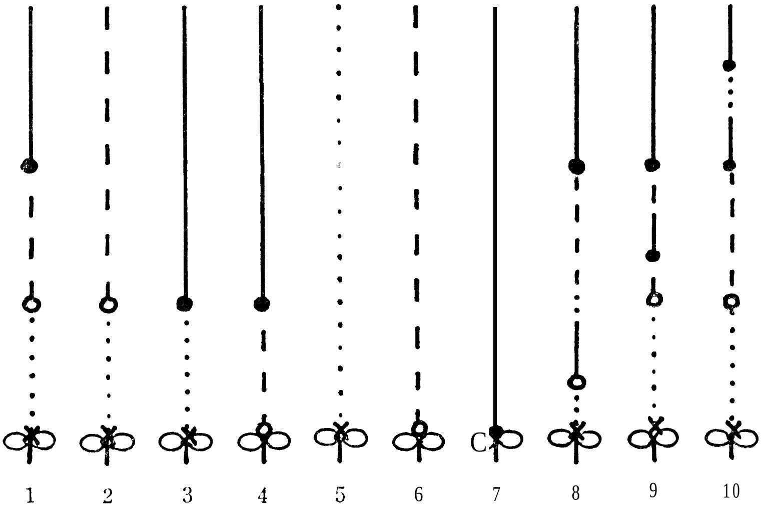

Fig. 10. Schematic representation of various types of sex expression induced by changing the balance of two reaction systems with photoperiodic treatments. 1 : control, 2 : P-phase, 3 : M-phase, 4 : S-phase. 5 : P- and M-phase, 6 : P- and S-phase, 7 : M- and S-phase are lost by treatment, respectively. 8 and 9 : female differentiation and 10: male differentiation, are promoted on the phases which are expected to be S-, M- and P-phases, respectively. See Fig. 3 and text.

tion. Low night temperatures, however, brought about growth inhibition and male flower formation (Table 5 and Figs. 7 and 8). Results on the effect of night temperature did not show that the lower the night temperature was, the more the female flowers were or the less male flowers (unpublished). In Exp. 4, the higher the day temperature was, the more blind nodes appeared. When night temperature, however, was 32 ℃ in continuous light, the blind nodes were most frequent (Nitsch et al., 1952, reported the blind nodes as underdeveloped flower nodes. See Fig. 3).

Concerning the sex differentiation, therefore, there may be an unknown substance which receives the external stimuli and directs the sexuality of flowers by changing the balance of the two reaction systems, one of which leading flowers to females and the other to males. Growth substances may be the derivatives of this unknown substance, or may be additively effective one on the sex differentiation and may induce elongation or inhibition.

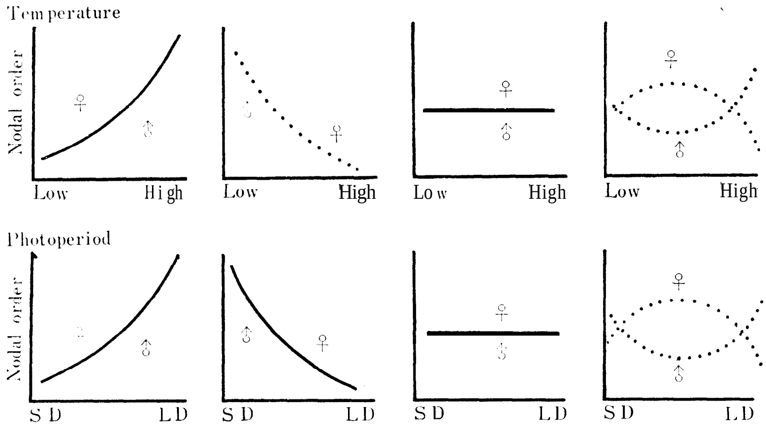

Fig. 11. Sensitivities to the temperature and the photoperiod for sex differentiation in cucumbers.

Various responses to photoperiods were summarized in Fig. 5 and schematically represented in Fig. 11. In most of the varieties SD promotes female differentiatiation (Type 1). In a few varieties the sex differentiation is not affected by photoperiod (Type 3). Variety " Higan-fushinari " is thought to be the Type 2 in which LD promotes female differentiation. This was confirmed by Exp. 2. As shown in Tables 3 and 4, lowering the temperature induces the increase of female flowers. This may be compared with one of the characteristics of flowering, that is, decreasing of critical daylength with lowering the temperature.

Fujieda (1965) thought that "Higan-fushinari" is insensitive to daylength from the results of his fieldworks. It may be natural, because when this variety is grown in spring, low temperature promotes female differentiation, but when grown in summer, LD promotes it.

Responses to temperature were summarized in Fig. 4 and shematically represented in Fig. 11. Most varieties could be divided into two types ; Type I : low temperatures promote female differentiation. Type III : sex differentiation is quite insensitive to temperature. However, Type IT whose varieties promote female differentiation at higher temperature, has not yet been reported so far. Saitô (1960) discovered, however, the existence of Type IV-A. This means that the female differentiation is greatest at a certain definite temperature, and that the lower or higher the temperature is, the less the number of female flowers are.

As the variety used by Saitô is thought to belong to Type **I,** further studies will remove most varieties of Type I into Type IV-A. At the same time this suggests that "Higan-fushinari" may be Type IV-B. The author suggested in Exp. 3 that the decreasing of the critical dark periods will occur for male differentiation with lowering temperatures and he also in Exp. 4 observed that the day temperature is more effective on sex differentiation than the night temperature.

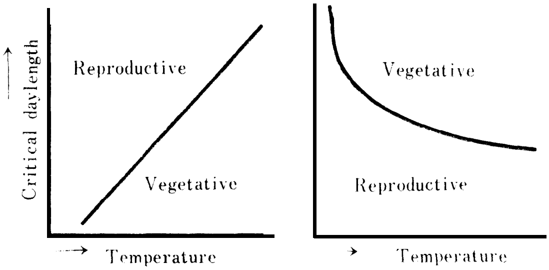

Fig. 12. Schematic representation of relationships between critical daylength and temperature on the growth in LDP (Left) and SDP (rsight) (After Esashi, 1965).

The above consideration will bring about one important question. Is there the photoperiodism in the sex differentiation like in the flowering ?

Garner and Allard (1923) discovered that not only the photoperiods but also temperatures are concerned with the photoperiodic responses in flowering. Further studies on flowering have brought about that the flowering response of SDP is greatest at a certain temperatue, and it is inhibited at the higher or lower temperature (Hamner and Bonner, 1938, Parker and Borthwick, 1939, Went, 1957), and that at lower temperatures critical daylength of SDP increases (Esashi, 1965), and that of LDP decreases (Lang and Merchers, 1943). (Fig. 12). Hamner and Bonner (1938) and Parker and Borthwick (1939) stated that the daytemperature is important for LD responses and night temperature for SD responses. In this case, a certain temperature is supposed to be most favourable for the dark process, and this temperature is called "optimal nycto-temterature." In general, for flowering it is known to be about 20 °C (Esashi, 1965).

Eguchi (1937) claimed that the photoperiodism of flowering must be discussed as the combination of the photoperiodic responses in flower primordia initiation and in flower development. *Cestrum nocturnum* (Sachs, *1956)* is one of the examples which require different photoperiodic conditions for flower initiation and for flower development. Photoperiodism has become to be found not only in flowering but also in various kinds of growth responses, such as tuberization (Esashi, 1959–1966), sprouting of tubers (Esashi, 1961–1966), resting-bud formation and its break on trees (Wareing, 1956), etc. With plants having hermaphroditic flowers, therefore, photoperiodism must be discussed on the flower primordia initiation and on the following developmental growth of flower organs as proposed by Eguchi (1937). With the monoecious plants, such as *Cucurbitaceae,* photoperiodism in the sex differentiation may be added to the above (Fig. 13).

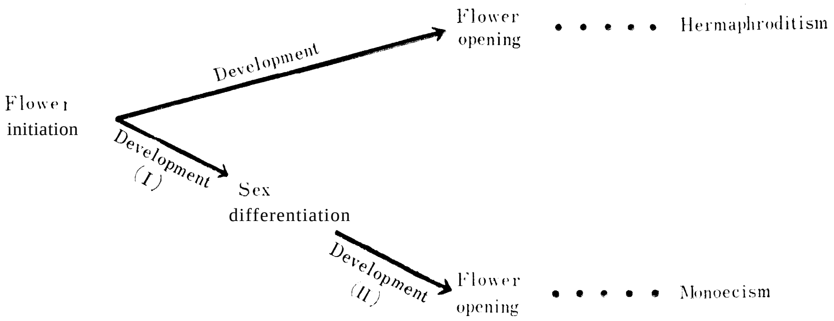

Fig. 13. The scheme of flowering processes in plants.

The following facts have been obtained with the sex differentiation in cucumbers : (1) LD promotes female differentiation in "Higan-fushinari" (Exps. 1 and 2). (2) The critical daylength for female differentiation in "Higan-fushinari" will be reduced by lower temperatures (Exps. 3 and 4). (3) For female differentiation in "Higan-fushinari" the daytemperature is much more effective than the night temperature. (4) There is an optimal nyctotemperature for female differentiation (Saitô, 1960).

These facts will suggest that there exists the photoperiodism in the sex differentiation of cucumbers. It is well known, however, that not only photoperiods and temperatures but also the plant age, sensitivity to stimuli, light intensity and quality are concerned with the photoperiodism. It would be premature, therefore, to conclude on the photoperiodism in sex differentiation in cucumbers. This problem will be treated in some detail in the following papers.

Summary

1. Cucumber varieties were grown under the combination of temperature and photoperiodic conditions in the Phytotron attached to Biotron Institute, Kyushu University.
2. As it has been known, low temperatures promoted female differentiation in most varieties. Some varieties, however, were insensitive to temperature conditions.
3. SD promoted female differentiation in most varieties. Some varieties were insensitive to photoperiodic conditions. With one variety "Higan-fushinari," however, LD could promote female differentiation.
4. Variety "Higan-fushinari" bore much more female flowers at lower temperatures.
5. Critical dark period for male differentiation in "Higan-fushinari" was about 9 hours at the temperature of 30-33 °C.
6. Female differentiation in "Higan-fushinari" was much more affected by daytemperature than night temperature.
7. Growth of seedlings was most prominent under the condition of 32 °C in the daytime and 25 °C at night.

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