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Studies on the photoperiodic sex differentiation in
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Aging effect on the photoperiodic de-
pendency of sex differentiation"

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After the discovery of photoperiodism in flowering of tobacco plant (Garner and Allard, 1920), during about 40 years, studies on the photoperiodic responses in plants have made clear that most kinds of plant growth are controlled photoperiodically and also that not only photoperiod and temperature but also light quality (Borthwick, Hendricks, Parker, *et al.*, 1948, 1950, 1959, Stolwijk, 1954, Esashi, 1963, 1966, Esashi and Oda, 1966), light intensity (Borthwick and Parker, 1938, Naylor, 1941, Jones and Bailey, 1956, Esashi, 1961, 1965), and plant age (Borthwick and Parker, 1938, Long, 1939, Esashi, 1963) are concerned with photoperiodism.

In the previous paper, the authors has got an insight that there might exist photoperiodism on the sex differentiation in cucumber (Matsuo, 1968). That is, in "Higan-fushinari" short day (SD) promoted the male differentiation and long day (LD) promoted the female one. The other fact was that even if plants were grown in SD condition, they bore female flowers after a certain period of growing. This means that plant becomes to bear female flowers on the upper distal nodes on the main stem, even if grown in SD condition. This fact brought about a problem how much the female differentiation becomes to be dependent on SD with the progress of aging. That is, with aging, in other words, when plants grew older gradual feminization has been obtained throughout the experiments. Therefore, even if the number of female flowers increased, when a long duration of LD induction was given to plant, it is impossible to decide whether such feminization was brought about

by the daylength treatment or by the aging. To solve this problem, it must be known whether the sex differentiation is affected by a short duration of photoperiodic treatment. This is one of evidences which can show that the sex differentiation is dependent on photoperiod.

On the other hand, it may be thought that the female differentiation is not dependent on LD, and the male differentiation will proceed, in turn, when SD stimulus is not produced. This situation will mean that feminization depends on the plant growth and that the nutrition and/or growth hormones are directly concerned with sex differentiation. It is well known in the case of *Arisaema* and *Begonia* that female flowers are differentiated when the tuber is of rather big size and its C/N ratio is rather high, while male flowers are differentiated when the tuber is smaller in size, and its C/N ratio is rather low. It may be safely said in the case of *Arisaema* and *Begonia*, therefore, that LD condition is concerned with the sex differentiation through the increasing of C/N ratio because LD condition will accumulate much more carbohydrates than SD one. In such case the photoperiodic response is not dependent on the cyclic change of day and night but on the nutrition or hormonal condition. This phenomenon can not be taken as the real photoperiodism (Esahi, 1967).

To solve the problem whether the sex differentiation is determined by photoperiodism, by the accumulation of growth substances, or by changing of C/N ratio, it will be one of the basic projects to investigate how much the male flower formation and the female flower formation depend on daylength.

In this paper, therefore, the following items were carried out to clarify whether the sex differentiation is determined photoperiodically as the flower formation or done nutritionally and hormonally as in the cases of *Begonia* and of *Arisaema*; (1) Relationship between aging and responsiveness of sex differentiation, (2) number of SDs and LDs required for photoperiodic induction in sex differentiation, (3) relative greatness of SD response to LD response induced by photoperiodic treatments.

The authors want to express their gratitude to Dr. Kunimitsu Fujieda, for his supplying seeds of cucumber with a good grace.

Materials and methods

Seeds of cucumber "Higan-fushinari" and "Sagami-hanjiro" were supplied by Dr. K. Fujieda, Oita Agric. Tech. Center, Usa, Oita Pref., Japan. They were germinated in darkness at 33°C. Each

seedling was transplanted into a 10×10 cm plastic pot, filled with sand, and was watered with Hyponex 1000 times solution. Seedlings were grown either in continuous light (LD 24: 0) or in short-day (LD 9: 15) until they were served for experiments. Temperature of room from 8: 30 A.M. to 5: 30 P. M. was kept at 30°C and the rest was kept 25°C . From 6 to 8 plants were used for each plot of experiments. After the treatment for several days, plants were removed and grown under the same condition as before. All the experiments were carried out in the Phytotron, attached to the Biotron Institute, Kyushu University.

To denote the features of sex differentiation, the following items were adopted: Number of female nodes from the 4th to the 10th nodes on the main stem counted from the proximal end, and/or percentage occurrence of the female node on the main stem.

Experiments and results

Experiment I, Changes in SD and LD responsiveness with the plant age

"Higan-fushinari" was grown in continuous light at the air temperature of 30°C in daytime (from 8: 30 A. M. to 5: 30 P. M.) and of 25°C at night (from 5: 30 P. M. to 8 : 30 A. M.). Plants were treated with SD for 10 days at the different ages which were counted with number of unfolded leaves. After the treatment they were removed and grown under the same conditions as before.

Results were summarized in Table 1 and Fig. 1.

Ito and Saito (1957) with variety "Sagami-hanjiro" observed that when the photoperiodic treatment was given for a shorter period, if plants were at the age of 4 leaves, the effect was greatest, and at much earlier

Table 1. Effect of SD treatment given at various ages of cucumber seedling "Higan-fushinari."

Ages shown in number of unfolded leaves	Number of female nodes occurred between the 4th and the 10th nodes
0 (right after the cotyledons unfolding)	4.7
1	3.4
2	1.0
3	3.2
4	5.0
5	5.5
Continuous light	7.0

and later ages the effect decreased. With "Higan-fushinari," as shown in Table 1 and Fig. 1, male nodes appeared most frequently when the treatment was given at the age of 2 leaves, and they decreased with the progress of aging. The influence of SD treatment was clearly observed on the nodes from the 4th through the 12th. The nodes affected by the treatment became to be localized at lower position along the main stem, and showed ascending, in turn, towards the upper distal portion with aging.

Fig. 1. Effect of SD treatment on the sex differentiation of cucumber "Higan-fushinari." Numbers set in the figure designate the age of seedlings as shown in the number of unfolded leaves.

Experiment 2. Number of SDs and LDs required for the male and female differentiation

"Higan-fushinari" and "Sagami-hanjiro" grown in SD (LD 9 : 15) or LD (continuous light, LD 24 : 0) were used. Air temperature and supplemental light conditions were set as the same as in Experiment 1. With "Higan-fushinari" 5 or 10 SD cycles were given to seedlings grown in LD, and 5 or 10 LD cycles to those grown in SD at the age of 2 or 4 leaves. With "Sagami-hanjiro" the same kind of treatment was given

exclusively at the age of 4 leaves.

The results are shown in Figs. 2, 3 and 4.

With "Sagami-hanjiro" 5 days of SD or LD treatments took effect on the sex differentiation and there was no significant difference in the effect between 5 days- and 10 days-treatments. With "Higan-fushinari" 10 days-treatment took more effect on sex the differentiation than 5 days-one at the age of 2 leaves. At the age of 4 leaves there could not be noticed any significant difference in the effect between 5 days- and 10 days-treatments.

Experiment 3. Photoperiodic dependency of sex differentiation

As shown in Figs. 2, 3 and 4, with "Sagami-hanjiro" the greatness of the effect of SD treatment to the LD grown seedlings was quite the same as that of LD treatment to the SD grown seedlings. With "Higan-fushinari" it was almost the same as with "Sagami-hanjiro" at the age of 2 leaves. At the age of 4 leaves the SD effect seemed to be superior to the LD effect.

Discussion

Change of responsiveness in photoperiodic induction with aging

Ito *et al.* (1954), Fujii *et al.* (1955) and Sekiya (1957) observed that

Fig. 2. Effect of LD and SD treatments on the sex differentiation in cucumber "Sagami-hanjiro." Daylength treatments were given to seedlings at the age of 4 leaves.

the sex differentiation occurred at first on the 3rd or 4th node on the main stem and it was followed by the occurrence on the upper and the lower nodes. As the flowers, whose sex is not fixed, are put or exposed under the influence of photoperiodic treatment, the portion where the effect of treatment is brought about is concerned with the time of treatment, that is, with the age of plants at which the treatment is given. For instance, if the treatment is given at the age when the sex on the 3rd or 4th node is going to fix, its influence will be observed on the nodes situated around the 3rd and 4th nodes, the influence becomes to be seen on the upper and lower nodes of the 3rd and 4th ones.

Fig. 3. Effect of LD and SD treatments on the sex differentiation in cucumber "Higan-fushinari." Treatments were given at the age of 2 leaves (A) and of 4 ones (B).

As mentioned previously, Ito *et al.*, Fujii *et al.* and Sekiya observed that the sex determination is most advanced on the 3rd and/or the 4th nodes. One of the authors (1968) confirmed that the blind nodes shifted upwards at higher temperatures and that the development of flowers became to be most advanced on the first and second nodes. Fujieda *et al.* (1965) observed that flower opening advanced on the lower nodes at lower temperatures. These facts will suggest that the position of node where the flower development and sex differentiation took place at first showed definite variation according to environmental conditions. The growing conditions of seedlings tested, therefore, were fixed in this series of

the experiment. As shown in Figs. 1 and 4, the treatment at the age of 2 leaves affected the stem position of 4412th nodes, and such affected portion by the treatment was shifted towards both lower and upper nodes with the progress of aging. The influence of treatment has been diminished and became to be lost at last. The same tendency had been obtained also by Ito and Saito (1957) and Minamikawa *et al.* (1958) ; when a short duration of temperature and photoperiodic treatment was given at the different age of seedlings after the expansion of cotyledons. When the treatment was given at the age of 20-25 days old after cotyledon expansion, when plants had been provided 2-4 leaves, the

Fig. 4. Effect of the photoperiodic treatments on the sex differentiation in cucumbers.

influence was greatest. No influence was observed, when the plants were treated not only just after the expansion of cotyledons but also at much advanced stages of growth. Peterson and Anhder (1960) and Fujieda (1963) observed male flowers on the nodes corresponding with the age of plants which were treated with gibberellic acids. It is wonder, however, if the same degree of male flower formation will be expected by the GA application of the same concentration as they used, when the plant age advances much more.

According to the data by Ito *et al.* and the present authors, the relationship between the plant age, when plants were treated with a short cycles of temperature and/or photoperiodic treatments, and the localization of nodes where the influence of such treatment was observed will be summarized schematically as in Fig. 5.

Fig. 5. Schematic representation of changes of responsiveness to environmental conditions for the sex differentiation in cucumber (Explanation is given in the text).

The curve (1) shows the relationship between the plant age when plants were treated and the nodal order where the influence of treatments appeared. The curve (2) shows the relationship between the treated age of plant and the nodal order where the sex of flowers was not affected by the treatment because of the advanced age of plant. The influences of photoperiodic treatments, therefore, could be obtained as the area enclosed by two curves, (1) and (2).

There is no effect of the treatment at the age $0 \rightarrow A$, because such young seedlings do not show any sensitivity to external stimuli.

The effect of treatment at the age $A \rightarrow B$ is expected on the area (a).

When the treatment is given at the age $B \rightarrow C$, the effect will be obtained as the areas (b_1) and (b_2) . When plants pass the age B, their sex determination will begin at nodes situated around B.

If the treatment is given at the age $C \rightarrow D$, the effect will be taken into count only with the area of (c) . Because the sex of each flower on the lower proximal nodes has been already determined before the age C.

There can not be observed any effects of treatment at all after the age D.

Thus, the influences of treatment could be observed with the nodes situated on the stem between 0 and D, when plants were treated at their age A-D. A rather short duration of treatment could not affect the sex expression when it is given either before the age A or after the age D. The responsiveness of the sex differentiation to environmental factors increased gradually until age B and after that it definitely decreased with aging.

Thinking of the sex expression in cucumbers (Fig. 5), both "Higan-fushinari" and "Sagami-hanjiro" bore female flowers on nodes from the proximal to D_1 seemed to be controlled more or less easily by environmental factors. Under the uniformity of conditions, sex is determined along the curve (1). If it is SD condition, "Sagami-hanjiro" bears female flowers, and "Higan-fushinari" bears male flowers, and *vice versa* if it is LD condition. When plants were treated with a rather short duration of treatment at the age A-D, the influences appeared as represented by the area enclosed by two different curves (Fig. 5). If the SD treatment was given to "Sagami-hanjiro" seedlings grown in LD, female flowers appeared on the nodes whose localization is corresponding to the plant age when the treatment was given. In the case of "Higan-fushinari," grown in LD, SD treatments caused male differentiation on the nodes localized corresponding to the treated age. On the contrary, in the case of SD grown seedlings, LD treatments caused female differentiation. The age D in "Higan-fushinari" was thought to be of 4-5 leaves (Exp. 1). As seen in Fig. 4, however, there occurs a tendency that the nodal order D, is just a little bit higher when LD treatments are given to SD seedlings than when SD treatments are given to LD ones. This may suggest that the SD dependency of sex differentiation is greater than LD one at the age D, and as the result, male flowers, i.e., SD response, appear (Fig. 1) when SDs are given to LD seedlings at the age of 4 or 5 leaves (Figs. 1 and 4), though there appear no effects of LD treatments on SD seedlings (Fig. 4). At lower temperatures, flower development and sex differentiation advance towards lower proximal nodes. This may suggest that the area in the figure will change to some extent according to growing conditions.

Thus, the responsiveness of sex differentiation to photoperiods and temperatures becomes to be greatest at a certain age, and it decreases before and after that age and the sexuality inclines to femaleness with the advance of aging (Fig. 6).

Fig. 6. Schematic representation of the relationship between plant age and sensitivity to external factors and responsiveness of female differentiation and of flower formation.

This may be compared with the changing of responsiveness and increasing of flowering ability with aging. That is, when SDPs receive SDs and LDPs receive LDs, they could respond to these photoperiods. This is called as the photoperiodic induction, and it is well known that these photoperiodic stimuli are received by leaves, especially young ones (Borthwick and Parker, 1938, 1939, Hamner and Naylor, 1939, Hamner and Long, 1939). The ability that plants respond to the photoperiodic stimuli, i.e., responsiveness increases with aging in general. *Kalanchoe* and soybeans being originally SDP, they can flower even in LDs as they age (Parker and Borthwick, 1939, 1940, Heinze *et al.*, 1942). This is because the reproductive growth response which acts against the vegetative growth response increases with aging.

In the case of sex differentiation, the following may be proposed ; There can not be detected any responsiveness at first. With aging, however, the effect which promotes male differentiation increases, and it precedes the effect of female promotion at the younger age. And the latter becomes to overcome the former with the progress of plant age.

Esashi (1963) suggested with *Begonia* that the destruction of stored substances in the tubers can increase the SD responsiveness, and also that it will come about possibly through the agency of immature leaves.

That is, the immature leaves could inhibit the responsiveness to SDs. Such an inhibitory action of immature leaves have hitherto been observed in soybeans (Fisher, 1955), cocklebur (Lincoln *et al.*, 1956),

Kalanchoe (Muscik, 1959), etc. It may be a common phenomenon in SDPs. On the other hand, Saito and Ito (1961) studied in detail with cucumbers the action of leaves on the sex expression using "Sagami-hanjiro" which promotes femaleness under SD conditions. They confirmed that immature leaves inhibit female differentiation, and thought that this inhibitory action of immature leaves is due to the action of auxins. In "Sagami-hanjiro" growth response and auxin contents are small and scarce under conditions as SDs and low temperatures which promote female differentiation (Ito and Saito, 1960). These facts will support the possibility that auxins have a certain positive connection with sex differentiation. Matsuo (1968) with "Higan-fushinari," however, observed that stem elongation did not always take place along with the male differentiation. This may suggest that all the development of flowers and their sex differentiation are not explained by the condition of auxin and other growth substances.

In "Higan-fushinari," the influences of temperature and photoperiodic treatments were clearly manifested on the differentiation of male and female flowers. On the other hand, in "Sagami-hanjiro," they revealed the variation in occurrence of the first female node, but not in the number of female nodes. These results will safely support that it may be the best way to evaluate effects of treatments to use the frequency of male or female nodes for "Higan-fushinari" and the location of the node with the first female flower for "Sagami-hanjiro." Therefore, in the present series of studies, in the case of "Higan-fushinari," seedlings were treated for 10 days at the age of 2 or 3 leaves and the results were discussed upon number of male or female nodes occurred from the 4th through the 10th nodes on the main stem. In the case of "Sagami-hanjiro," seedlings were treated for 10 days at the age of 4 leaves and the effect was denoted by the variation in occurrence of the first female node.

Number of photoperiodic cycles

It is well known that cucumbers bear female flowers after a certain period of growing has passed, that is, they have a tendency of gradual feminization as they age. This gives us a question to what extent the male differentiation in "Higan-fushinari" and the female one in "Sagami-hanjiro" are dependent on the SD condition. This means that if plants are treated with a certain temperature and photoperiod for a number of days, for instance, throughout the experiment, it is quite impossible to tell whether the sex differentiation is due to those treatments or to the progress of aging. Therefore, to know that the sex differentiation is dependent on photoperiod, it is necessary to clarify if male or female differentiation is promoted by the photoperiodic treatment for a very short duration.

It has been known in flower formation that a few cycles of photoperiod can cause photoperiodic induction, and that the increase of number of cycles could force the flowering and increase the number of flowers (Garner and Allard, 1923, Borthwich and Parker, 1938, Hamner and Bonner, 1938) and also that the less number of cycles was necessary to induce the same photoperiodic induction as they grew up older (Long, 1939, Yoshida, 1960).

Using "Sagami-hanjiro" Ito and Saito (1957, 1960) studied the number of SD cycles which could induce the female differentiation. They disclosed that only 2 cycles of SD can cause lowering of the site of first female node and an increasing of number of female nodes, and that effectiveness of SD treatment enlarged with the amount of SD cycles given. With "Sagami-hanjiro," the authors did not found out any differences in the appearance of the first female node and also in the number of female nodes occurred between 5 and 10 cycles of SD (Figs. 2 and 4). In "Higan-fushinari," however, the effect of 5 cycles of SD was greater than that of 10 cycles of SD, when seedlings were treated at the age of 2 leaves. When they were treated at the age of 4 leaves, there could not be found any differences between those 2 kinds of SD treatments. This situation may be caused by the fact that the female flower formation by SD treatment at the age of 4 leaves was rather slight in amount as compared with that at the age of 2, because the tendency of such female flower formation showed a definite increase with aging.

Photoperiodic dependency of sex differentiation

In the above, it was confirmed that the tendency of female flower formation increased with age not only in "Higan-fushinari" but also in "Sagami-hanjiro," and that the sex differentiation could be controlled by several cycles of photoperiodic treatment.

As it may be thought that the young flowers which are under hermaphroditic state are directed to develop into male or female one as the result of a certain antagonistic reaction between the LD response induced by LD and the SD response induced by SD, it must be useful to clarify which of SD and LD responses is greater than the other.

In "Higan-fushinari," the relative amount of SD response (male flower formation) and that of LD one (female flower formation) was almost the same at the age of 2 leaves, but at the age of 4 leaves the former became to be greater than the latter (Figs. 3 and 4). This was also indicated in the observation that the lowest node of Pistillate phase was located at higher portion when LD grown seedlings were induced by SD than when plants were grown up in SD condition throughout the experiment (SD control).

In general, plants show a tendency of making transition to reproductive stage without difficulty as they age. Concerning this, it is well known that there are distinguished three different types of daylength requirement. Plant belonging to the first type show a qualitative daylength requirement, never losing its requirement though it shows definite decrease in amount, and continue its vegetative growing so long as the ordinary temperature is prevailing (begonia and cocklebur). Plants of the second type are those having a certain requirement, and they become to lose their daylength requirement with age, and enter into reproductive stage even under photoperiods unfavourable for the reproduction (soybean, rye and wheat). Plants of the third type are those called as day-neutral plants which have no photoperiodic requirement for their reproduction from a relatively early stage just after germination (tomato and sunflower). The differentiation of these types is very significant because of its assumed relation to the mechanism of differentiation of ecological varieties of such as rice and soybeans (the transition of type I into type III corresponds to the evolution of the early mature variety from the late mature variety (Esashi, 1963).

There have been known a number of varieties in cucumber. Fujieda (1966) classified them into the following 4 types of sex expression according to the ability to bear female flowers;

- (1) Monoecious type: Some flowers are pistillate and some others staminate.
- (2) Mono-gynoecious type: The proximal part of the main stem shows the monoecious and the upper distal part the gynoecious condition.
- (3) Gynoecious type: The flowers are usually pistillate, but a few staminate ones are produced under a certain definite environment.
- (4) Andro-monoecious type: Some flowers are hermaphrodite and others are staminate.

If the word "flowering" is substituted by the word "feminization" from the view point of the easiness of transition from maleness to femaleness with age, it can be thought that the varieties belonging to monoecious type and to gynoecious type, except "MSU 713-5," are classified as the II type of flowering and that "MSU 713-5" as the III type, that is a day-neutral form.

In the previous paper, Matsuo (1968) stated with "Higan-fushinari" that LDs promoted female differentiation, the critical daylength for male differentiation was about 15 hours at 30-33°C, and that lower temperatures promoted female differentiation. In Exp. 1, the authors could confirm that female formation increased with age, sex differentiation was affected by several cycles (extending only a short duration)

of photoperiodic treatments, greatness of the SD response and that of the LD one at the age of 2 leaves were almost the same, but at the age of 4 leaves the former was just a little bit greater than the latter.

From these results it can be duly concluded that there is a photoperiodism in the sex differentiation of cucumbers like in the flower formation in plants. In consequence they indicate that "Higan-fushinari" and "Sagami-hanjiro" will be taken as LDP and SDP for female differentiation, respectively.

Summary

1. Using cucumber varieties, "Higan-fushinari" and "Sagami-hanjiro," the experiments were carried out in the Phytotron to know the aging effect on the sex differentiation and the effect of SD and LD treatments.
2. Aging effect was examined by using the seedlings grown in continuous light and being treated with SD for 10 days at the different ages and observing the male nodes on the main stem which were brought about by SD treatment.
3. The influence of the treatment at the age of 2 leaves appeared at the portion extending from the 4th through 12th nodes along the main stem, and that decreased, in turn, with age and only a little influence could be observed when the treatment was given at the age of 5 leaves.
4. The portion of stem where influences of treatment were brought about did not always shift upwards along the main stem, but it lowered along the main stem from the upper part where the development of flowers advanced most.
5. The effect of number of cycles of photoperiodic treatment given and the effectiveness of LD or SD cycles were examined using SD- and LD-grown seedlings. "Higan-fushinari" was treated with SDs or LDs for 5 or 10 days at the age of 2 or 4 leaves. "Sagami-hanjiro" was treated with SDs or LDs for 5 or 10 days at the age of 4 leaves.
6. In "Sagami-hanjiro," no difference in the effect between 5 and 10 days treatments was observed. In "Higan-fushinari" the effect of 10 days treatment was greater than that of 5 days one at the age of 2 leaves. At the age of 4 leaves, however, the difference between them was not clear.
7. Effectiveness of SD treatments and that of LD ones were the same in "Sagami-hanjiro" and in "Higan-fushinari" at the age of 2 leaves. In the latter form at the age of 4 leaves, however, effect of SD treat-

ments was greater than that of LDs.

8. From these results, considering together with those of the former paper, it is concluded that there could observe the photoperiodism in the sex differentiation of cucumber like in the flower formation in plants.

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