Flower Initiation of the Dodder, Cuscuta japonica in Total Darkness on Artificial Culture Medium

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I. Introduction

It was reported by LOO[8] that the dodder, *Cuscuta campestris* can be grown aseptically on artificial medium which contains, besides agar-agar, only sugar and minerals. No addition of juice or extract of natural sources is required for the growth of this parasitic plant. In this condition it was also capable of initiating flowers under illumination.

Recently it became known that some long day[3,9] or neutral plants[1,4,6,7] can initiate the flower primordia in total darkness, i. e. independently of light. This fact was also ascertained in some short day plants.[10]

In the present paper experiments were performed to reveal whether the dodder can also initiate flower primordia in the total darkness.

II. Material and Methods

For acceleration of germination the seeds were treated with concentrated sulphuric acid for 30 minutes and washed thoroughly with running water.[5] For sterilization they were immersed in 75 % alcohol for 5 minutes under low pressure, and shaken in a 10 % calcium hypochlorite for 25 minutes. Five sterilized seeds were sown per tube and placed in the complete darkness. The basic culture medium contained: $Ca(NO_3)_2$-0.2 g, $MgSO_4$-0.36 g, Na_2SO_4-0.2 g, KNO_3-0.08 g, KCl-0.065 g, KH_2PO_4-0.0165 g, $Fe_2(SO_4)_3$-a trace; Sucrose-50 g, Agar-10 g and dist. Water-1000 cc.

The test tubes were wrapped with black light-proof paper and placed in either warm or cool temperatures. The observation of flowering was carried out with a binocular microscope at about 40 days after the start of the experiment.

Cuscuta japonica var. *viridicaulis* seems to be a short day plant, as it is in flower from late summer to autumn. The initiation of the flowering of the lateral bud is promoted by the removal of terminal bud,[2] i.e. it seems that the flowering is retarded or prevented by the existence of a stem tip. Therefore in most experiments the flowering response was studied using the first lateral bud of a plant from which

the stem tip was removed. During the procedure of removing the terminal bud the darkened plants were unavoidably exposed to light for some 10-15 minutes.

III. Experimental Results

1. The first experimental treatments were started on April 28, 1953. The seeds were sown aseptically in test tubes, divided into five groups and placed under the following different conditions, 1) cool temperature, in darkness; 2) warm temperature, in darkness; 3) warm temperature, in darkness for fifteen days and after removal of the terminal buds the first lateral buds were cultured at a warm temperature in the darkness; 4) warm temperature, in darkness for fifteen days, and subsequently a cool temperature in the darkness; 5) warm temperature, in darkness for fifteen days, and then after the removal of the terminal buds the first lateral buds were cultured at a cool temperature in the darkness. The results are shown in Table I.

Table I.

Culture of *Cuscuta* seedlings in darkness, April 28 to June 7, 1953.

Temperature for the 1st 15 days	Temperature of the subsequent 25 days	Condition of the terminal buds	No. of plants	Flowering (%)
8 - 15° C	8 - 15° C	intact	8	0
25° C	20 - 26° C	"	9	0
25° C	20 - 26° C	removed	10	0
25° C	8 - 15° C	intact	12	0
25° C	8 - 15° C	removed	14	57.1

As shown in the Table I, it was evident that the flower primordia appear on the first lateral buds in the total darkness if the terminal buds of the seedlings were removed and cultured at a cool temperature, but the intact seedlings remained vegetative. At warm temperatures the first lateral buds did not initiate a single flower primordium although the stem tips were removed.

This fact reveals that for the flowering of the dodder in the darkness the terminal buds must be removed and the plant subsequently cultured at a cool temperature.

2. The second treatments were done from July 3 to August 25, 1953. The sterilized seeds were sown in test tubes on July 3 in complete darkness at 25° C. When the seedlings were 12 days old, they were at

least about 75 mm in length and the lateral buds were barely discernible with the naked eye. The stem section measuring about 2–3 mm long, bearing the first lateral bud was excised aseptically from the seedlings with sterile forceps in a sterilizing box. During the procedure the seedlings were exposed to light for 5–10 minutes. They were then cultured aseptically on the basic medium in 18 × 150 mm test tubes (Fig. 1), and placed under four different conditions, 1) in total darkness at a warm temperature; 2) in total darkness at a cool temperature; 3) under continuous light; 4) under short days consisting of 8 hours light and 16 hours dark periods at a warm temperature. The results are shown in Table II.

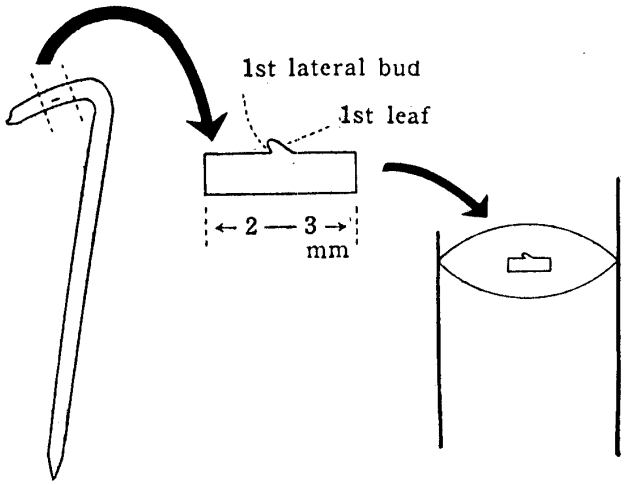

Fig. 1. Schema of the culture

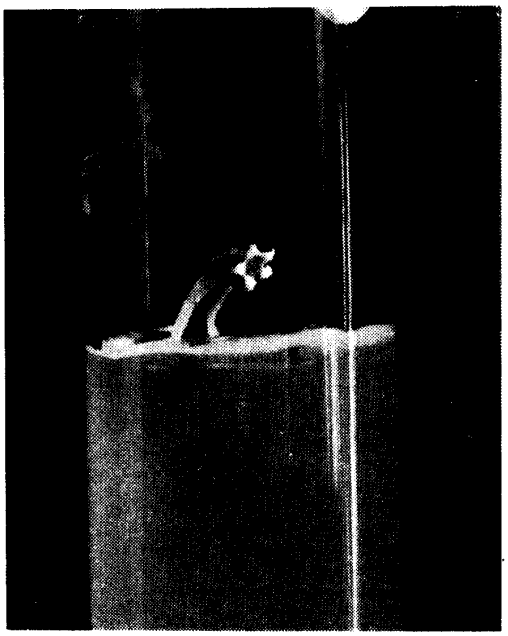

Fig. 2. Flower developed from excised 1st lateral bud of dodder *in vitro* by cool temperature in complete darkness.

From the Table II, it is obvious that the lateral buds of *Cuscuta* seedlings can be induced to initiate a flower primordium by the short day treatment or by cool temperature in complete darkness as reported for the *Pharbitis Nil*.[10] At cool temperatures the majority of the lateral buds produced flower buds in the complete darkness, whereas in the darkness at warm temperatures no flower primordia were formed. Therefore, the effect of cool temperature on the initiation of flower buds in the complete darkness is evident (Fig. 2).

Table II.

Flowering response of 1st lateral buds of *Cuscuta* under different conditions.

Condition		Flowering response*	Aveage position of nodes bearing the first flower	Aver. number of nodes developed in non-flowering individuals	Average length of lateral buds in cm.	Color of lateral buds
Light	Temperature					
darkness	25 – 29° C	0/21	—	4.1 ± 1.1	10.7 ± 1.0	yellowish white
"	10 – 15° C	18/22	3.6 ± 1.0	2.8 ± 0.5	2.1 ± 1.1	"
continuous light	25 – 29° C	0/20	—	4.8 ± 1.0	8.5 ± 1.0	green
8 hours short day	25 – 29° C	14/19	3.0 ± 1.3	3.0 ± 0.4	1.9 ± 1.0	"

* Denominator: Number of lateral buds examined.
Numerator: Number of lateral buds with flower buds.

3. The author reported that the unvernalized seedlings of the *Raphanus* can initiate a flower primordium in the darkness by adding ribonucleic acid to the culture medium.[11] An experiment was carried out to investigate the effects of ribonucleic acid on the flowering of *Cuscuta*.

The treatments were started on March 31, 1955. The sterilized seeds were sown and the stem section bearing the first lateral bud was excised on April 15, and cultured on the following culture media in the complete darkness at 25° C: 1) the basic medium was autoclaved for 20 minutes under a pressure of 1.5 kg/cm^2; 2) ribonucleic acid of 500 ppm was added to the basic medium and was autoclaved for 20 min-

Table III.

The effect of ribonucleic acid on the flowering response of the first lateral buds of *Cuscuta* in the darkness at 25° C. Observations of the floral development were made on June 2.

Medium	Method of sterilization	Flowering response	Average position of nodes bearing the first flower	Average number of nodes observed in non-flowering individuals
basic medium	autoclaved (pH. 5.8)	0/26*	—	5.1 ± 1.4
basic medium	autoclaved (pH. 5.4)	15/30	4.0 ± 1.0	4.2 ± 1.1
+ RNA 500 ppm	100° C (not autoclaved) (pH. 6.2)	0/31	—	5.8 ± 1.1

* Denominator: Number of lateral buds examined.
Numerator: Number of lateral buds with fower buds.

utes under a pressure of 1.5 kg/cm^2; 3) the same medium as the previous one (2) was sterilized twice for 30 minutes with steam of 100° C. The results are shown in Table III.

Flower primordia were not initiated either in the control lots or in the lots to which RNA was added and not autoclaved. In the lots of the autoclaved culture medium containing RNA; 50 % of the treated plants initiated flower primordia. From the results it seems obvious that the promoting action on the flowering may be due rather to a substances formed by the destruction of ribonucleic acid by autoclaving under a pressure of 1.5 kg/cm^2, rather than to ribonucleic acid itself.

IV. Summary

1. *Cuscuta* seedlings are capable of initiating a flower in lateral buds when the seedlings from which the terminal buds were removed are cultivated under sterile conditions in complete darkness at a cool temperature.

2. The excised first lateral buds of *Cuscuta* seedlings which were aseptically cultivated on a basic medium in test tubes, can be induced to initiate flower buds by short day treatment, or by a cool temperature in total darkness.

3. Some substances derived from ribonucleic acid by autoclaving seems to promote the flower initiation of the lateral buds of the *Cuscuta*.

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