5. Development of Flowering Stalks in *Silene Armeria* in Relation to Auxin Metabolism

(Preliminary Report)

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Most of the long day plants keep their rosette form under short day conditions but rapidly shoot up into flowering stalks under long day treatment. In the present investigation the author tried to explain the mechanism of this phenomenon, called "bolting", from the point of view of auxin metabolism.

In floricultural practice, when sown in autumn, *Silene Armeria*, a long day plant, remains in rosette form throughout the winter, then shoots into a flowering stalk and begins to blossom next spring and produces seeds in early summer. When sown in spring, it shoots into a flowering stalk and blossoms in the very same season without passing through the rosette stage. When the plant is artificially kept under short day conditions, it never shoots and blossoms, but remains in the rosette form. But even under short day conditions, when the temperature becomes higher, the plant can attain a considerable height owing to an increase of number of nodes. However, this growth is different from bolting which is caused by the elongation of internodes and is irreversible, i.e. when it once begins it cannot be suppressed by any means. Growth due to internode elongation also occurs when the plant is bred in a light of low intensity. Here the growth is not irreversible, i.e. the rosette form is reinstated when the plants are subjected to a light of sufficient intensity. In such a case a plant with two rosettes, separated by the nodes with elongated internodes, is obtained. Thus there are three types of growth in *Silene Armeria*: the first is the rosette type under short day conditions which grows by increasing the number of nodes; the second is the irreversible bolting type which is caused by the elongation of internodes brought about by long day treatment; the last is the etiolation type which is also caused by an elongation of internodes but it occurs only when the light is not strong enough. These types are determined by light conditions and cannot change from one to another either by application of solutions or lanolin pastes of Indole-3-acetic acid (IAA) and α -Naphthalene acetic acid (NAA) in various concentrations, or by other treatments such as nipping the apical bud, cutting off leaves, etc.

Experiments were carried out, in order to analyse from the point of the phytohormone concept the difference between the rosette type developed at high temperature and the bolting type. The plants used were raised in the field as well as in the greenhouse, and were subjected to suitable photoperiodic and thermal conditions which made the simultaneous use of both types possible.

1) Amount of Auxin in the Shoot

Various forms of auxin are known to occur in plant tissues, and the method of isolation and assay is different according to the form of auxin concerned.⁹⁷⁾¹¹⁾ In the present investigation various methods were tried in estimating the auxin content of various parts of the plants in both types of growth. It was difficult in winter or early spring to obtain the diffusible auxin from the stem of either of the two types. It was, however, always obtainable from the stems of flowering plants in the later season. Concerning the extractable auxin, its amounts extracted from the tissues of bolting plants by various methods were not always larger than those from the rosette plants. The results of the experiments were so ambiguous that no certain correlation could be found between the amounts of diffusible or extractable auxin and the growth of the plants. This would be due to the rapid consumption of the auxin for the growth in the bolting plants.

2) Diffusible Auxin from Stem Sections Obtained by the Application of Synthetic Auxins to Cut Apical Surfaces

By another method it was experimentally established that there is a definite difference between the two types of growth. Stem sections, 5 to 20 mm. in length, were cut from the bolting and the rosette types. An agar block ($2 \times 2 \times 2$ mm.) containing IAA (100 γ /ml. to 0.01 γ /ml.) was put on the upper apical surface of each of the stem sections, and the auxin diffused into an agar block attached to the basal surface of the section was assayed by means of *Avena* test after 1 to 3 hours. The auxin concentrations were supraoptimal for the *Avena* coleoptiles in higher concentrations than 0.5 γ /ml. as shown in Table I. In the rosette type the negative curvature caused by the auxin diffused to the lower agar block was at its maximum when the section was treated with 100 γ /ml. IAA and it was decreasing with the application of lower concentrations of IAA. Thus IAA applied to an apical surface seems to be able to diffuse only with difficulty into the lower agar block. On the contrary in the case of the bolting type the negative curvature reached its maximum at 5 γ /ml. and was decreasing with increasing concentration. Here the lower agar block seems to have contained auxin in supraoptimal concentration so

Table I

Curvature of *Avena* coleoptile in response to the auxin diffused into the lower agar block on application of agar blocks containing IAA on cut upper surfaces of stem sections of *Silene Armeria*

IAA conc. in the upper agar block (γ /ml.)	0	100 (No block)	50	5	0.5
Rosette type	- 7.2	-22.0	-10.0	- 3.8	0
Bolting type	-10.1	- 1.8	- 2.2	-23.8	-16.8
Curvature by the upper agar block itself	—	- 5.0	-10.5	-17.0	-19.5

that the curvature was highly reduced. From these facts it may be concluded that the amount of auxin diffused into the lower agar block may be larger in the bolting type than in the rosette type.

The same results were found also with NAA. The difference in the diffusibility of auxin between the two types of plants, however, may depend on the difference in morphological structure, i.e. more nodes really existed in the stem sections of the rosette plants than in those of the bolting plants. But experiments which are reported in the following suggest that the above-mentioned results could be explained in the terms of a destruction or inactivation of auxin in the stem of the rosette plants.

3) Activity of Converting Tryptophane to Auxin in the Squeezed Shoot Sap⁽³⁾⁽⁴⁾⁽⁸⁾⁽⁹⁾⁽¹⁰⁾⁽¹²⁾

The shoots were first frozen in dry-ice and then crushed. The pulp obtained was then filtered through a cloth and centrifuged. The activity of converting tryptophane to auxin was tested in three receptacles as follows: the first contained 1 ml. of the filtrate and 2 ml. of 0.01 M phosphate buffer (pH 6.8), and the second contained, besides the same amounts of filtrate and buffer solution, 1 mg. of l-tryptophane. The contents of the third receptacle were the same as those of the second, except that the filtrate had been previously boiled. All the receptacles were incubated at 22°C for 4 hours. After the incubation each mixture was brought to pH 2.8 with 15% tartaric acid solution and extracted three times with ether. The gathered ether extract was shaken three times with 1 ml. of a saturated glucose solution containing 8 per cent sodium bicarbonate in order to bring the acidic substances into an aqueous solution. The aqueous fraction was adjusted to pH 2.8 by tartaric acid and immediately extracted by ether, and the obtained ether fraction was evaporated to dryness. 1 ml. of water was then added to each fraction and stirred well. Agar blocks were soaked in the solutions thus obtained and used for the *Avena* test. As represent-

ed in Table II, the leaf extract from the bolting plants showed stronger activity in converting tryptophane to auxin than that from the rosette plants.

Table II

Enzymatic conversion of tryptophane into auxin by the leaf juice of different growth types of *Silene Armeria*. The amount of auxin formed is represented by degrees of curvature of *Avena coleoptile*.

Leaf juice		Not heated		Heated
Tryptophane		Not added	Added	Added
Juice from	Rosette type	0	- 8.2	-3.0
	Bolting type	-2.1	-31.3	-7.9
	Bolting type+rosette type	—	-28.2	—
	Bolting type+heated juice of rosette type	—	-38.6	—

In order to see whether the lower activity of the extract from the rosette plants is due to some inhibitor of the auxin forming enzyme system present in this type of plants, the extract from the bolting plants was mixed with the extract from the rosette plants. But as shown in Table II, no decrease in the auxin formation was found. So it can be concluded that the leaves of the rosette plants have a lower activity in converting tryptophane to auxin.

4) Inactivation of IAA by Acetone Precipitate from the Squeezed Shoot Sap

Concerning the inactivation of IAA, an enzyme system which causes the oxidation of IAA has been found in etiolated pea seedlings.^{1,5,6)} In order to compare the two types of plants regarding the effectiveness in destroying IAA, the IAA oxidizing enzyme was isolated by a procedure similar to that of Galston and Bakers.¹⁾ The juice obtained in the manner as described above was precipitated by the addition of 4 ml. of acetone per 10 ml. of juice. The precipitate was centrifuged, and suspended in the same volume of acetone. This was stirred well and the precipitate was centrifuged. The acetone was decanted and discarded. This washing procedure was repeated three more times. The precipitate was then suspended in a cold phosphate-citrate buffer of pH 6.6 of half the volume of the original juice and left overnight.

This was then centrifuged and the clear supernatant liquid was used for the test. All the above-mentioned procedures were carried out chiefly below 0°C, or a little above that when necessary. The liquids were obtained from the bolting and the rosette plants

Fig. 1. The velocity of IAA destruction

The reaction mixture had a total volume of 4 ml., consisting of 2 ml. of test solution plus 2 ml. of 50 γ per ml. IAA. At the time indicated, 1 ml. of reaction mixture was sampled and the disappearance of IAA was followed by means of Salkowski's colorimetric technique. Temperature 25°C, in darkness

mixtures of the extracts and IAA were incubated in the light (Mazda filament lamp, about 3000 Lux at the surface of reaction mixture) as well as in the dark, in order to determine the effect of light upon the IAA destruction in the mixture. But there was no difference in the velocity of IAA decrease due to light. On the other hand, the IAA destruction was completely inhibited in the crude sap from the green tissues, unwashed with acetone, in light as well as in darkness.

5) Conclusion

The experimental results so far obtained show that in the bolting plants much more auxin is formed and at the same time less of it is destroyed than in the rosette plants. It may be assumed that this is the cause of the rapid elongation of stem in

by the same procedure. To each of them, the same volume of 50 γ per ml. solution of IAA was added and incubated at 23°C to 25°C. At intervals indicated in Fig. 1, 1 ml. of incubated reaction mixture was sampled and mixed with 4 ml. of Salkowski-Tang's reagent. After heating for 15 minutes over a boiling water bath, the resultant pink pigment was extracted with 1 ml. of iso-amylalcohol. The photo-absorption was measured with a photoelectric colorimeter using a green filter, Mazda V-GI.

The results showed that the extracts from the shoots of both bolting and rosette plants destroyed IAA, in contrast to the reports that the IAA destroying enzymes are present only in etiolated tissues or roots and not in green tissues.⁵⁾⁶⁾ The velocity of the IAA destruction was much higher with the extracts from the rosette plants than that from the bolting plants, as shown in Fig. 1. Hence it can be inferred that the bolting occurs because more auxin is produced and less of it is destroyed. The

the bolting plants.

As it is due to the difference in the ability of formation and destruction of auxin whether a plant will bolt or remain in the rosette stage, it may be imagined that an exogenous application of synthetic auxin would not necessarily induce bolting.

Auxin is believed to be an essential factor in the growth of plants. The correlation between growth and the assayed auxin level has been a subject of eager investigations, however, it has been only in some cases—not always—ascertained. The present experiments have succeeded to explain the stem elongation in terms of the formation and the destruction of auxin, instead of merely stating the amounts of auxin. Thus the experiments point the way toward an explanation of the rapid growth of shoots in bolting plants in the light of auxin metabolism.

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