

## Contents of Embryo Factor in Developing Seeds of *Datura* and *Lupinus*

By

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### I Introduction

It was reported that the contents of gibberellin-like substances<sup>1)2)</sup> and kinetin-like substances<sup>3)</sup> changed in the course of development of seeds and fruits in several plants. The largest content of the latter was found at the stage of the most active cell-division in fruits, indicating some significant physiological relation between the kinetin-like substance and fruit growth.

It was reported that young *Lupinus* seed contained embryo factor, a growth-promoting factor for the young embryo<sup>4)</sup>. To study the physiological relation of embryo factor to the growth of the embryo in the seed, it is necessary to examine the contents of the embryo factor in developing seeds and other tissues during the development of the fruit, the seed, and the embryo.

### II Materials and Methods

Ethanol diffusates from seed, placenta and fruit coat of *Datura tatula* and from seed, "endosperm" (including the tissues of endosperm, nucellus and integument), embryo and pod of *Lupinus luteus* were tested for their growth-promoting activity for excised *Datura* embryo. Preparation of the crude diffusate was the same as described previously<sup>4)</sup>. The crude diffusates were decolorized with charcoal, and added to the basal media composed of White's solution<sup>4)</sup>, 40g sucrose and 9g agar per liter. The media were autoclaved at 1.0 kg/cm<sup>2</sup> overpressure for 10 minutes. Young embryos ranging between 0.2 and 0.35 mm in length were isolated from the seeds as described by van Overbeek et al.<sup>5)</sup>, and cultured on media to be tested. Test tubes, each with 6 ml of the medium and one embryo, were maintained at 30°C in darkness for 5 days. The growth of the embryos was expressed with "growth value" (final length/initial length), and relative contents of embryo factor in the tissues were represented by the differences in growth value between control and test lots.

### III Results

i) Experiments with *Datura* diffusate: The growths of the fruit, the seed and the embryo were shown in Figs. 1, 2, and 3. Within 5 days after pollination, the fruit and the seed started to grow. Several days later, rapid increases in size and fresh weight of them were observed. Embryos in the seeds, however, were still in globular stage (0.05~0.1 mm length) or in heart-shaped one (about 0.2 mm in length), and after further 2 or 3 days a rapid growth of the embryo was observed with the elongation of the cotyledons and the hypocotyl. In three weeks after pollination, the thorns of fruit coat became harder and the seed coat turned brown, the fruit and the seed developed into the largest size and weight, and the embryos approached to full size of 7 mm.

On the 8th, 12th, 16th and 20th days from the time of pollination, each 6.25 g of fresh seeds, having 1375, 740, 619 and 579 seeds respectively, was harvested, and examined by means of excised

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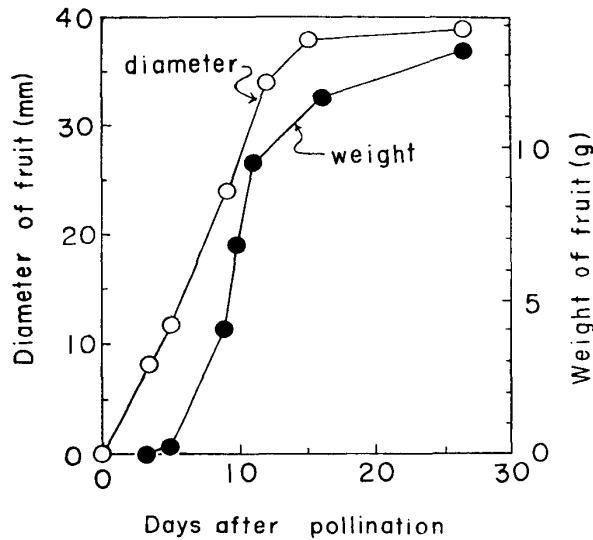


Figure 1. Growth of *Datura* fruit.

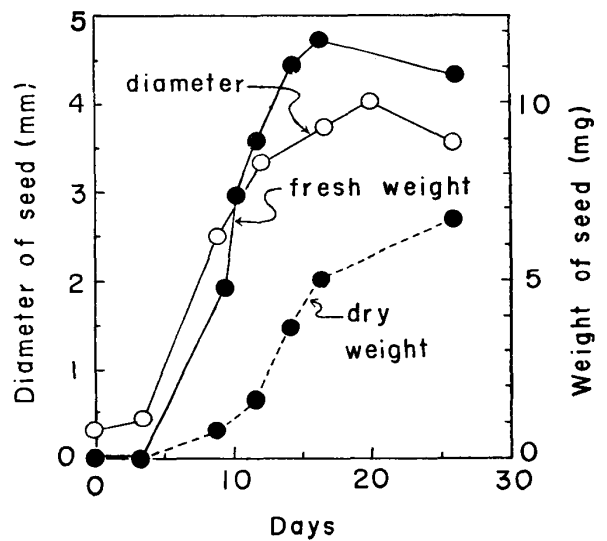


Figure 2. Growth of *Datura* seed.

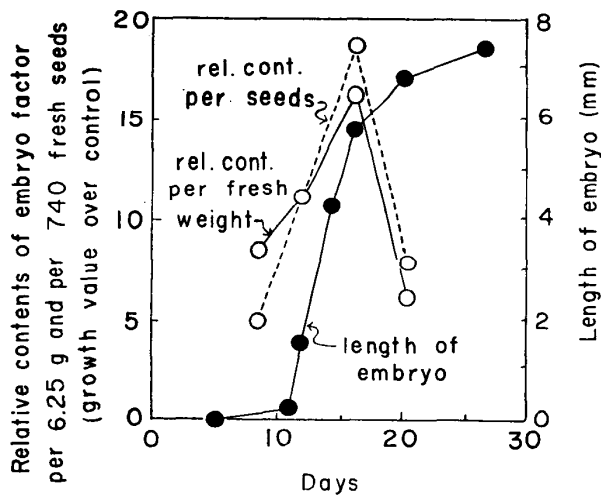


Figure 3. Length of *Datura* embryo in the seed and the relative content of embryo factor in developing *Datura* seeds. Relative content was represented by the difference in growth value of excised *Datura* embryo between control and experimental lots. Solid line; cultured on the media containing the diffusate from 6.25 g fresh seeds per 100 ml. Broken line; calculated from the above value on the basis of 740 seeds.

embryos (Fig. 4).

The content of the factor in 6.25 g seeds was much higher in the young seeds than in the old ones. The highest content was found on the 21st day, at which the most rapid growth of the seed was observed, and the embryo started to grow rapidly (Fig. 5). The maximum fresh weight of seed was observed on the 26th day, when dry weight was still increasing. Thereafter, the factor decreased, reached the minimum content on the 35th day, and increased again in the mature seed.

When the relative content per 6.25 g seeds were calculated on the basis of 32 seeds at the corresponding stages, the young seeds contained a very little amount (Fig. 4). Increasing with seed growth, the content per 32 seeds reached the maximum on the 28th day. At that stage the seed also has the maximum fresh weight. Later the content decreased.

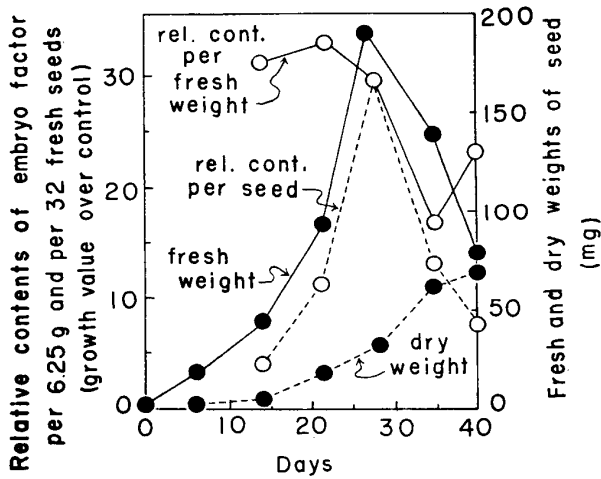
To learn the embryo factor in the seed more in detail, the contents of the factor in embryo

*Datura* embryos (Fig. 3). After the 8th day, the content began to increase with rapid growth of the seed and in advance of the embryo growth. Further increase of the factor was observed, followed by more rapid embryo growth. The highest content was found in 16 day-old seeds. Thereafter it decreased abruptly with seed ripening and slowing down of the embryo growth.

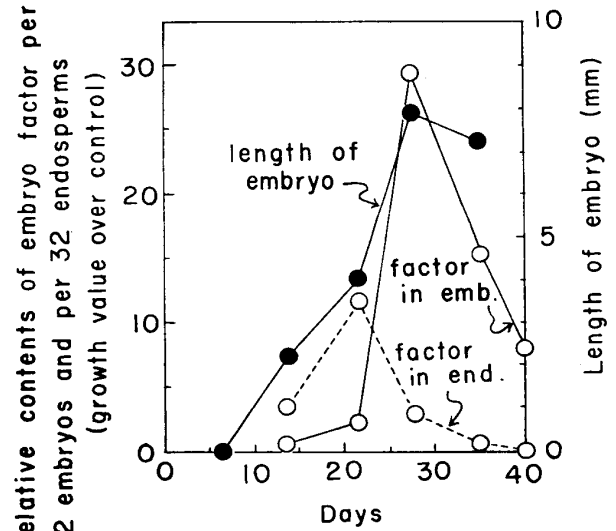
When the relative contents of the factor in 6.25 g seeds were calculated on the basis of 740 seeds, a similar curve with the maximum content on the 16th day was obtained (Fig. 3).

The embryo factor activity in placenta and fruit coat was also examined. In both tissues, the maximum content, though considerably less than that in the seed, was found also on the 16th day.

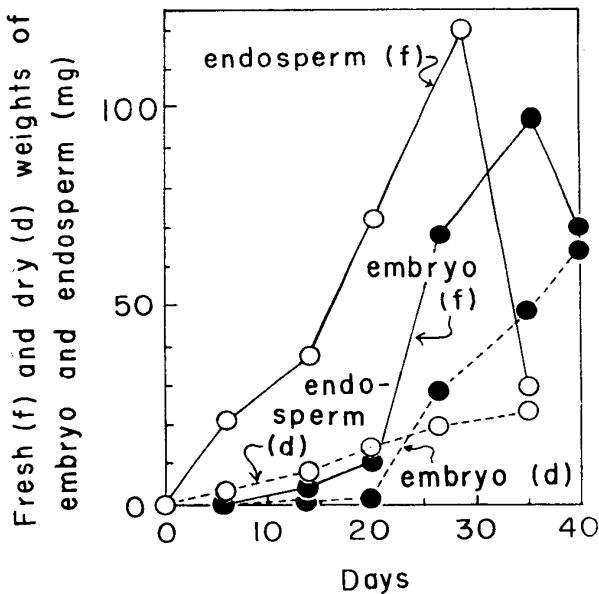
ii) Experiments with *Lupinus* diffusate: Growth curves of seed in fresh and dry weights were shown in Fig. 4. Each 6.25 g of *Lupinus* seeds, having 221, 97, 32, 40 and 81 seeds respectively, was harvested on the 14th, 21th, 28th, 35th and 40th days from the time of pollination. Each ethanol diffusate was tested with *Datura*



**Figure 4.** Growth of *Lupinus* seed and relative content of embryo factor in it. Relative content was represented by the difference in growth value of excised *Datura* embryo between control and experimental lots. Solid line; cultured on the media containing the diffusate from 6.25 g fresh seeds per 100 ml. Broken line; calculated from the above value on the basis of 32 seeds.



**Figure 6.** Growth of *Lupinus* embryo and relative contents of embryo factor in 32 embryos and endosperms. Relative content was represented by the difference in growth value of excised *Datura* embryo between control and experimental lots. *Datura* embryos were cultured on the media containing the diffusates from 32 embryos and endosperms per 100 ml.



**Figure 5.** Growth of *Lupinus* embryo and endosperm.

of the factor and a rapid enlargement of the embryo started. On the 28th day the maximum content and the largest size of embryo were attained. With the continuous increase in fresh and dry weights and slight decrease in size of the embryo, the content of the factor in the embryo decreased. A considerable amount of the factor was recognized in fully mature embryos.

It seems that the maximum content of the factor in "endosperm" stimulates the early development of the embryo, and the larger content of it in embryo tissue accelerates the growth of the embryo itself.

and "endosperm" and their growths were examined separately (Figs. 5 and 6). Fresh weights of "endosperm" and embryo were maximum on the 28th and 35th days respectively, and thereafter both decreased rapidly, while dry weight of them increased progressively.

To determine the content of embryo factor, 32 embryos and "endosperms" were harvested on the 14th, 21th, 28th, 35th and 40th days after pollination. On the media containing the diffusates obtained from the materials per 100 ml, *Datura* embryos were cultured, and the differences in growth value between control and test lots were observed. The factor in "endosperm" increased up to 21 days from the time of pollination. Thereafter it decreased to an immeasurable amount, in spite of the continuous growth of the "endosperm". On the other hand, the factor in the embryo was in smaller amount, when the factor in "endosperm" was maximum. Thereafter, a rapid increase

In young pod of *Lupinus*, the factor was also found in considerable amount, while it was scarcely found in old yellowing pod.

#### IV Discussion

In *Datura* and *Lupinus* the content of the embryo factor in each seed began to increase two or three days after the start of a rapid growth of the seed. Increasing factor which was produced in the seed seemed to stimulate the start of the growth of the embryo. Further several days later a rapid growth of the embryo occurred. The stage of the maximum content of the factor in a seed agreed with that of the largest growth of the embryo. On the other hand, when the content of the factor on the basis of fresh weight of seed, corresponding to the concentration of the factor in the seed tissue, was examined in various stages, much higher concentration of the factor was found in younger stages of the seed. This was especially intensified in the case of young *Lupinus* seed. The rapid growth of the young embryo is maintained by such higher concentration of the factor in seed. The younger embryos in vitro require higher concentration of the factor<sup>6)</sup>.

When *Lupinus* seed was examined separately in the "endosperm" and the embryo, the factor in the former increased in the early stage, but soon decreased in spite of the continuous growth of the "endosperm". Thereafter, the factor in the embryo increased rapidly with the development of it. In the early developmental stages the factor is supplied to the embryo from the endosperm, and in later stage it may be produced in the tissue of the embryo and stimulates the growth of it. A large amount of the embryo factor was found also in the tissue of mature embryo of *Sechium edule*<sup>4)</sup>. Similar embryo factor was found in the placenta and the fruit coat of *Datura* and the young pod of *Lupinus*. The factor found in such tissues other than the embryo may stimulate the growth of them.

Thus, it is highly possible that embryo factor is identical or similar to kinin which stimulates the growth of various tissues in vitro. It must be studied whether or not embryo factor activity is caused by plant kinin or its analogs.

#### V Summary

1. In the seeds of *Datura tatula* and *Lupinus luteus*, the content of embryo factor increases with the development of the seed. A rapid growth of the embryo occurs two or three days after a rapid increase of the factor.

2. The highest content of the factor is observed at the time of the most active growth of the embryo.

3. In the older seeds the content of the factor decreases, but in the fully mature *Lupinus* seed a considerable amount of it is found.

4. The embryo factor is found also in placenta and fruit coat of *Datura* and in pod of *Lupinus*.

The authors are indebted to Prof. S. Imamura, Kyoto University, for his guidance and counsel during the course of this study.

#### VI References

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