

## **STUDY ON THE EXPERIMENTS ON MAIZE MESOCOTYL GROWTH BY LENGTHENING**

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### Abstract

Aim of the present study is to show a desensitizing action, maybe a density-dependent inhibition, of high dilutions 30°, 200° CH, dynamized or non-dynamized, of a plant hormone, the indoleacetic acid (IAA). At this purpose, three experiments on the growth by lengthening of maize mesocotyl (specific test for the dosage of substances with an auxinic action) have been performed. Data obtained show that pre-treatments with centesimal solutions of IAA 30° CH, 200° CH, both treated and non-treated at 100° C, induced a significant growth decrease compared to the treated sample.

**KEY WORDS:** IAA 30°-200° CH; maize mesocotyl growth.

### Introduction

Indoleacetic acid (IAA) is considered the most important auxin from a physiological point of view, and it is involved in many physiological phenomena and in limited functionally specific actions: cellular extension, cellular division, and some processes of differentiation, including its role in root growth.

The optimum concentration of IAA in the plant tissues ranges from 10<sup>-4</sup> and 10<sup>-6</sup> M, but its effect has also been noted at doses of 10<sup>-10</sup> M.

The segments of maize mesocotyl, pre-treated with highly diluted solutions of IAA 30°-200° CH dynamized, treated at 60°/100° C for 30 minutes, induced a significant inhibition of growth by lengthening; it is due that to "contact inhibition" (density-dependent inhibition) provoked by pre-treatments of 30°-200° CH IAA on auxin receptors?

It is not a question of chemical information but, maybe, it is a question of electromagnetic information on molecular receptors.

That is an hypothesis to verify.

### Material and methods

Seeds of F1 Plenum Dekalb hybrid maize, class 600, have been washed in running water for about 20 hours and then planted in tanks containing vermiculite. The tanks have been placed in the dark for 5-6 days at 25°C.

From the apical portion of the mesocotyl of each seedling, a 9.5 mm-long segment has been excised. These segments (10/plate) have been placed in Petri dishes, each one containing 50 ml of solution to be tested (see the tests outlines).

The dishes have been kept in the dark, at room temperature, for 24 hours. Any growth in length induced by each solution in exam, compared to those obtained in the incubated control samples, has been taken as the activity value of these solutions. All experiments were conducted "blind", and data concern average of final growth of segments by lengthening by examination after 48-62 hours.

Data obtained have been statistically processed by the variance analysis and the Student-Newman-Keuls test, and the average for each dish has been used for analysis.

Outline of the tests performed in the 1th experiment.

For each test, six plates with fourteen segments each have been analyzed, for a total of 50 replica/test.

1. Pre-treated IAA 30° CH(1)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM (10<sup>-4</sup> M);

2. Pre-treated IAA 30° CH dynamized(2)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

3. Pre-treated IAA 30° CH(3)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

4. Pre-treated IAA 30 CH dynamized(4)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

5. Pre-treated control(5)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

6. Pre-treated control dynamized(6)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

7. Control

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

8. Control dynamized

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

9. Treated IAA

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

10. Treated IAA dynamized

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

(1) Five hours before in a solution of IAA 30° CH

(2) Five hours before in a solution of IAA 30° CH dynamized

(3) Five hours before in a solution of IAA 30° CH/treated at 60°C for 30 minutes

(4) Five hours before in a solution of IAA 30° CH dynamized/treated at 60°C for 30 minutes

(5) Five hours before in H<sub>2</sub>O

(6) Five hours before in H<sub>2</sub>O dynamized

Outline of the tests performed in the 2th experiment.

For each test, five plates with ten segments each have been analyzed, for a total of 50 replica/test.

1. Pre-treated IAA 30° CH(1)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

2. Pre-treated IAA 30° CH(2)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

3. Pre-treated IAA 200° CH(3)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

4.Pre-treated IAA 200° CH(4)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

5.Pre-treated control(5)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

6.Pre-treated H<sub>2</sub>O(6)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

7.Control

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

8.Treated IAA

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM.

- (1) Five hours before in a solution of dynamized IAA 30° CH;
- (2) Five hours before in a solution of dynamized IAA 30° CH/treated at 100°C for 10 minutes;
- (3) Five hours before in a solution of dynamized IAA 200° CH;
- (4) Five hours before in a solution of dynamized IAA 200° CH/treated at 100°C for 10 minutes;
- (5) Five hours before in dynamized water;
- (6) Five hours before in dynamized water + IAA 100 uM.

Outline of the tests performed in the 3th experiment.

For each test, five plates with ten segments each have been analyzed, for a total of 50 replica/test.

1.Pre-treated IAA 30° CH(1)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

2.Pre-treated IAA 30° CH(2)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

3.Pre-treated IAA 200° CH(3)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

4.Pre-treated IAA 200° CH(4)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

5.Pre-treated control(5)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

6.Pre-treated H<sub>2</sub>O(6)

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

7.Treated IAA

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0 + IAA 100 mM;

8.Control

Phosphate buffer 10 mM + sucrose 30 mM pH 6.0;

- (1) Five hours before in a solution of dynamized IAA 30° CH;
- (2) Five hours before in a solution of dynamized IAA 30° CH/treated at 100°C for 10 minutes;
- (3) Five hours before in a solution of dynamized IAA 200° CH;
- (4) Five hours before in a solution of dynamized IAA 200° CH/treated at 100°C for 10 minutes;
- (5) Five hours before in dynamized water;
- (6) Five hours before in dynamized water + IAA 100 uM.

## Results

### First experiment

The results of the first experiment are listed in TAB. 1.

Different letters show significant differences at  $p = 0.01$ .

Data obtained show that:

A. Treated sample and treated dynamized showed a growth similar of 2,5 mm compared with the control;

B. Pre-treatments with solutions of IAA (30° CH sample, 30° CH dynamized, 30° CH treated at 60°

C, 30° CH treated at 60° C dynamized) induced a significant growth decrease compared with the control and with the pre-treated control.

TAB. 1 - Mesocotyl average length (mm) measured after 48-62 hours.

6 Pre-treated control dyn

10.48

7 Control

10.51

8 Control dyn.

10.51

5 Pre-treated control

10.63

4 Pre-treated 30° CH dyn. at 60° C

11.62

3 Pre-treated 30° CH at 60° C

11.67

1 Pre-treated 30° CH

11.96

2 Pre-treated 30° CH dyn

12.05

9 Treated

12.97

10 Treated dyn  
13.13

### Second experiment

The results of the second experiment are listed in TAB. 2.  
Different letters show significant differences at  $p = 0.05$ .

Data obtained show that:

- A. The differences observed are highly significant ( $p < 0.05$ ).
- B. The treated sample, compared with the control, showed a growth of 5.3 mm approximately.
- C. Pre-treatments with dilutions of IAA (30° CH, 200° CH), both treated and non-treated at 100°C, induced a significant growth decrease compared with the treated sample.
- D. Pre-treated 6 (pre-treated in H<sub>2</sub>O and then placed in a solution of IAA 10<sup>-4</sup> M) showed a growth similar to that of pre-treated 1, 3, 4, but lower compared with the pre-treated 2 (level of the test significance:  $p = 0.05$ ).

TAB. 2 - Mesocotyl average length (mm) measured after 48-62 hours.

5 Pre-treated control  
10.44

7 Control  
10.55

3 Pre-treated 200° CH  
12.35

1 Pre-treated 30° CH  
12.45

4 Pre-treated 200° CH 100°C  
12.52

6 Pre-treated H<sub>2</sub>O  
12.57

2 Pre treated 30° CH 100°C  
13.11

8 Treated sample  
15.88  
Third experiment

The results of the 3th experiment are listed in TAB. 3.  
Different letters show significant differences at  $p = 0.05$ .

Data obtained show that:

- A. Treated showed a growth of 4,5 mm compared with the control.
- B. Pre-treatments with dilutions of IAA (30° CH, 200° CH treated and non-treated at 100° C) induced a significant growth decrease compared with the control.
- C. This decrease is significant in pre-treatments 2 - 3 - 4 compared with pre-treated 1.
- D. Pre-treated 6 showed a significant growth compared with pre-treated 2 - 3 - 4 but it is smaller than pre-treated 1 (significance level:  $p = 0.05$ ).

TAB. 3 - Mesocotyl average length (mm) measured after 48-62 hours.

8 Control  
10.33

5 Pre-treated control  
10.36

4 Pre-treated 200° CH at 100° C  
11.52

2 Pre-treated 30° CH at 100° C  
11.68

3 Pre-treated 200° CH  
11.77

6 Pre-treated H<sub>2</sub>O  
12.07

1 Pre-treated 30° CH  
12.46

7 Treated  
14.8

Discussion

To be emerged from these results are some discrepancies relative to the problem of using dynamized IAA, and to that concerning the possibility of rendering inactive the dilutions with the heating to 100° C for 30 minutes.

From this data it seems that the problem of using dynamized or non-dynamized IAA not have any importance for the result, and as a matter of fact, it is known that the technics that have prepared the homeopathic dilutions of IAA, agitated always in the flasks, also for the "dilutions non-dynamized". That is to say, in practice, the dynamized preparations have been shaken more numerous and forcefully of that of the non-dynamized.

From all the experiments the result is that the dilutions 30°-200° CH of IAA, provoke a significant reduction of growth in respect to samples treated with IAA 10-4 M.

We used in the experiments 100 mM IAA dynamized and non-dynamized to see if there is some difference of action shaking.

This happened both utilising the dilutions dynamized and non-dynamized and heating both at 60° or at 100° C.

It appears not to confirm the hypothesis that the heating above 60° C inhibits the effect of the homeopathic dilution. These results each demonstrate an activity of inhibition of the growth also with dilutions heated at 100° C for 30 minutes.

The samples in H<sub>2</sub>O dynamized utilised in these experiments are produced with 100 agitations, as used in the classical preparation of the homeopathic remedies.

The preparation of the samples of potentized IAA have been prepared respectively with 30 and 200 passages only in distilled water, the solution has been additioned after in five plates for each test altogether to the phosphate buffer 10 mM + sucrose 30 mM pH 6,0.

Only in the third experiment was there a discrepancy with the sample n. 6, pre-treated in water, which has given the same positive result with the samples pre-treated with 30°-200° CH of potentized IAA.

We have seen after that the sample n. 6 has been prepared utilising the same flask for the solution of IAA 10-4 M. The technical operator has more time to rinse the flask, obtaining perhaps as IAA dynamized with Korsakov's preparation.

## Conclusion

This research confirms the activity of a high potency of a substance as the auxin, in our case, which unfolds notoriously its actions in doses of 10-4 / 10-6 M.

The IAA in 30°-200° CH does not reveal the same roll of the auxin on the growth, but provokes a form of desensitization on the biological target of the phythormone.

According to the experimentation of huMan hormones, to high dilutions as follicolinuM, progesterone, insulinuM, thyroidinuM, adrenalinuM, or as amine biogene as hystaminuM, which permits a control of the biological effects, here the usage of infinitesimal dosages of IAA allows an inhibitory control on the growth. It has nothing to do with an enzymatic inhibition, since the homoeopathic dilutions do not have an active pharmacological molecule, but it could be a hypothetical question of a density-dependent inhibition.

The research on the high dilutions has the necessary criteria of protocol different from the traditional research. The activity of the high potency is to be demonstrated on a vegetable or animal tissue, only if preceding treatment of a pharmacodynamic substance of which it is known experimentally the receptors on which it affects and/or the biological effects which it produces. Our analysis of data confirms the action of high dilutions of a phythormone, IAA, compared to control samples.

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