Biological Models for Research in Homoeopathy - Berlin Journal on Research in Homoeopathy

Synergism of Action Between Indoleacetic Acid (IAA) and Highly Diluted Solutions of CaC03 on the Growth of Oat Coleoptiles

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Summary
This work was conducted to assess the effect of highly diluted solutions of CaC03 on the growth of oat coleoptiles stimulated by 100 ZM of indoleacetic acid. Ten hours before application of the hormone, the coleoptiles were pretreated with homoeopathically prepared CaC03 solutions. Pretreatment with a potency of 5° CH (homeopathic dilution) resulted in a statistically significant increase in growth as compared with coleoptiles treated with indoleacetic acid (IAA) alone.
Key Words: CaC03, indoleacetic acid (IAA), homeopathic synergism

Introduction

The biological regulation mechanisms which allow every unicellular or multicellular organism to coordinate its various cellular metabolic activities (e.g. nutrition, growth, differentiation, and reproduuotion) is strictly subordinate to a system characterized by three phases: stimulus, reception, and response. The nature of the slimulus can be physical (e. g. thermo- or photo-induction of a morphogenetic process) or chemical. The nature of receptiort is essentially less variable, since it is dependent on the protein structure and its informational character, which create specific responses:

a) recognition of a part of molecules,
b) modifi cati on of it s own configuration, and
c) adaptation of its own activity on the basis of the structural modifications that have taken place [1] .

Characteristics of the Hormones of Plants

One fundamental characteristic of plant hormones in the almost total absence of specific production sites, both at the tissue level and at the intercellular level. Only the phototropic response Of cereal coleoptiles can be ascribed to a specific auxin-producing site, which is localized in the upper part (apex) of the plant and which is responSible for the coleoptile growth of oats or wheat The characteristios of plant hormones can be described as follows:

1. The action of each hormone is multiple in that it is manifested both in growth phenomena (multiplication or extension) and in phenomena of differentiation.
2. Biological hormonal activity is made evident through its specific action in particular tissues and in different stages of development
3. From a qualitative and quantitative point of view, every hormonal response is variable with respect to two parameters: the concentration of the hormone and the specificity of the target tissue.

Consequently, many of the physiological processes of plants are under the control of not one single hormone, but of a system of different hormones. From this one can deduce the difficulties in studying plant hormones. Furthermore, the reception sites of the hormones, the sites of hormonal biosynthesis and the paths of degradation are still unknown [2].

The Physiology of Auxin

As previously said, each functional manifestation cannot be attributed to the exclusive action of one specific hormone. Gas chromatography and high-pressure liquid chromatography in conjunction with mass spectrometry have shown that various vegetable tissues contain different classes of hormones which are capable of influencing the activities of the tissues themselves.

The auxins, of whi ch ind oleaceti c acid (I M) i s con si dered the mo st important from a physiological point of view, appear to be involved in many physiological phenomena and in limited functionally specifi c acti on s. including cellular exten sion, cellular divi si on, and some processes of differentiation, including its role in root growth.

The optimum concentration for the most decisive action of IM in the plant tissues ranges from 10-4 and 104 M, but its effect on the lengthening of roots grown in vitro has also been noted at doses of 10-1° M [3] IM synthesis has been demonstrated in various ti ssue s. particularly in young ti ssue s with meri st ematic activity, e . g . the apici caulinari, the gemmae, the germinating seeds, and flowers and fruit in the initial phase of growth [4]

Research Objective

CaC03 was chosen since it can influence neodifferentiation, acting principally as a cellular stimulant by improving the reception of calcium ions from the culture medium.

From the relevant literature, Kirkby et al. [5,9] note that the movement of calcium in growing tissues takes place in the direction of the newly formed cells, and it is presumed that this migration is induced and stimulated by IM [5]. The objectives of this research project, drawn from this premise, were to investigate and evaluate the effects of different dynamized dilutions of CaC03 and to determine whether these dilutions exhibit any synergistic effect on the IM-induced growth of segments of oat coleoptile, even at infinitesimally small concentrations that significantly exceed the Avogadro's number.

Materials and Methods

Small oat plants were grown in the dark until the coleoptile had reached a length of 2-3 cm. Segments of standard length (about 3-5 mm) were isolated from the sub-apical region. Segments each were placed in Petri dishes containing the various test solutions and kept in the dark as described [6]. 6 Petri dishes with IO, segments each were used for every single condition. This amounts to a total number of 780 segments.

A. IAA induced growth of oat segments: Segments were placed in 10 mM phosphate buffer (pH 6.5) containing 100 ,uM of either dynamized or non-dynamized IAA. The controls were placed in 10 mM phosphate buffer (pH 6.5) or in 10 mM of dynamized phosphate buffer (pH 6.5) alone (Figures 1 and 5).

B. IAA induced growth of oat segments after pretreatment with different homeopathic dilutions of CaC03 (Figures 2 and 3). Ten hours before addition of IAA, the coleoptile segments were pretreated with dynamized CaC03 at concentrations of 5, 15 and 30 CH, respeotively. Separate batohes of segments were pretreated with these homeopathic dilutions, which had been heated to a temperature of 60°C and left to cool. All solutions were coded. Finally, 100 I1M (10-4 M) of IAA were added. The time courses of IAA induced growth after pretreatment with the various dynamized CaC03 solutions and their heated counterparts were compared to each other and to the controls The lengthening of the coleoptiles was taken as a measure of IAA activity and the synergistic action of CaC03.

C Comparison of lAA-independent growth responses af ter treatment with dynamized CaC03 . To study the effect of CaC03 on oat coleoptiles, one batch of segments were treated with homeopathically diluted CaC03 (5, 15 and 30 CH) without subsequent IAA treatment (Figure 4).

Results

Figure 1: The difference in length increase between the control specimens (control 1) and the dynamized control specimens (control 2), both of which were kept in 10 mM phosphate pH 6.5, was negligible. In both test groups, control and dynamized control, treatment with IAA resulted in a drastic change in the

growth rate. A growth increase of 15-24% was noted as ofthe second hour oftreatment, and the increase reached 35-45% by the ninth and tenth hour of treatment.
If the specimens treated with simple IAA were compared to those treated with dynamized IAA, an growth increase of 3-5% in the dynamized group was observed. Although this is not to be considered statistically significant, the hypothesis of an improved action with dynamized IAA is not to be excluded.
Figure 2: From the 6th hour onwards, the specimens that were pretreated with dynamized CaCO3 (5 CH) 10 hours prior to the addition of 100 pM IM achieved an growth increase of 8-9% more than the specimens treated with simple IM alone (see Fig. 1). Compared to the specimens treated with dynamized IM (see Fig. 1), the improvement in growth of the above-mentioned specimens was 5% more from the 6th hour, increasing to 10% more at the 7th hour, and then stabilizing at 4% at the last hour.

The specimens treated with dynamized CaC03 15 CH prior to addition of IM, achieved a greater increase from the 8th to the 10th hour as compared to those treated with IAA alone. This increase is not statistically significant. When compared with the data of the specimens pretreated with heated (60°C) CaCO3 5 CH and CaCO3 15 CH before the addition of IAA, the increase noted was 9%, and therefore of some, albeit limited, significance.
Figure 3: The specimens pre-treated with CaC03 30 CH prior to addition of IAA achieved an increase in segment growth of 3-5% as compared to the specimens treated with IM alone and an increase of 3-7 % if compared to the specimens pretreated with CaC03 30 CH that had been heated to 60°C. This growth differences, however, are not statistically significant.
Figure 4: The growth of specimens treated with CaC03 5 CH, 15 CH or 30 CH only was insignificant as compared to those treated exclusively with IAA.

Conclusions

From these data, it. is possible to draw various conclusions as well as a new hypothesis for study and research. The conclusions of this research can be summarized as follows:

a) The specimens pretreated with a dynamized 5 CH potency of CaCO3 prior to the addition of the growth hormone IAA exhibited a statistically significant increase in growth as compared to specimens treated with IAA alone.

b) This research confirms the hypothesis that homoeopathic dynamization is neutralized by heating to 60°C [7]. This can be seen in Figure 2, which shows that the growth obtained in the case of active dynamizations is 9% more than in the samples where the heat-treated solution was applied. Even though this result is not statistically significant, it might well indicate a tendency.

c) When applied exclusively (without IAA), dynamized 5, 15, and 30 CH potencies of CaCO3 have no effeot on the growth of coleoptile segments isolated from the subapical area of oats. CaCO3 therefore does not have an activity similar to that of auxin.

d) The action of dynamized CaC03 especially at 5 CH, is strictly synergistic and seems to augment the phyto-hormonal action of IAA.

Hypothetical Considerations

The hypothesis has been formulated that the effect of auxin on growth is largely the consequence of acidification of the extracellular space. The acidification effect induced by auxin seems to depend on the stimulation of transport of protons across the cellular membrane. The release of protons is linked to a stimulus of the velocity of uptake of cations, in particular of K+. The auxin stimulated exchange of H+/Ki seems to depend on a protein with ATPase activity [3]. The correlation between the stimulation of growth and the activation of the electrogenic exchange system of H+/K+ suggests that the acidification of the cellular walls plays an important role in the increase of the plastic extendability induced by hormones [2]. It can thus be hypothesized that the role of CaCO3 is that of:

a) increasing the extracellular Cat+ concentration, thus stimulating the auxinic activation of the eleotrogenic exchange system of H+/K+ [5] and

b) all owing a better uptake of calcium from the culture medium, con sequently augmenting the functional capacity of ions at the cellular level.
According to Kirby there is a close relationship between endogenous IAA and calcium ions [5,9].
This is validated by the observation that compounds such as TIBA, which are capable of obstructing auxin transport, also suppress Ca++ transport, thus causing symptoms typical of calcium deficiency [8].

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Note: This article was originally published in OMEOPATIA (No.2, 1991). We thank Dr. V. Bonifacio Vitale for the kind permission to print this English version.
Editor's Address: Prof. J. Hornung, Universitatsklinikum Steglitz,1000 Berlin 45, Germany, Tel.: (030) 834 3975