POLLEN PHYSIOLOGY AND FERTILIZATION

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EDITED BY H. F. LINSKENS

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Foreword

This volume contains the written version of papers delivered at the First International Symposium "Pollen Physiology and Fertilization" held at the Department of Botany, University of Nijmegen on August 29th–31st, 1963. The purpose in organizing this meeting was to bring together people specialized in studying those fundamental processes in higher plants leading to formation of the zygote – the basis of seed production and cereal and fruit crop plants.

An important part of the symposium were the discussions; they resulted in a valuable exchange of ideas and a breakdown of many misunderstandings formerly passed on in literature. The recorded discussions were subjected to some editorial abridgment after transcription from tapes and revised by each participant.

It is a pleasure to record the sincere appreciation of all participants to the speakers who contributed to the success of the conference. Special thanks are due to all members of the staff of the Botanical Laboratory of the University of Nijmegen for preparing the meeting. Grateful acknowledgment is made to Dr. C. J. M. AARTS, Administrative Director of the Faculty of Science of the University of Nijmegen and his collaborators for helpful assistance in organization of the symposium.

University of Nijmegen
(The Netherlands)
January 1964

H. F. LINSKENS

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1. Physiology of the embryo sac

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A HISTOCHEMICAL INVESTIGATION OF THE OVULES, EMBRYO SACS AND SEEDS IN SOME ANGIOSPERMS †

Embryology of the Angiosperms becoming an experimental science, establish its relations not only to genetics and plant breeding but to physiology and biochemistry as well. However, embryonic processes in plants are hitherto insufficiently studied from the physiological-biochemical aspect. The application of histochemical methods is one of the approaches contributing to the understanding of the physiological-biochemical essence of these processes.

In the recent years papers have appeared in the USSR dealing with histo- and biochemistry of embryonic organs in the Angiosperms. E. A. Britikov, N. V. Zinger, T. P. Petrovskaya-Baranova, N. N. Polunina, T. S. Kantor, E. N. Ustinova, V. A. Poddubnaya-Arnoldi and others are applying histochemical technique to the investigation of the developments of ovules, macrospores and embryo sacs, as well as to that of the pollen and pollen tubes in several representatives of the Angiosperms (sp.sp. *Rosa, Delphinium, Paeonia, Lilium, Ornithogalum, Zea, Taraxacum, Helianthus annuus, Linum usitatissimum, Panax ginseng, Onobrichis sibirica, Cypripedium insigne, Calanthe Veitchii, Dendrobium nobile* etc).

Localization of substances, enzymes included, in embryonic organs of various Angiosperms was taken under study at different developmental stages. Reactions to peroxidase, cytochromeoxidase, polyphenoloxidase, dehydrases, catalase, phosphatases, proteins, nucleic acids, ascorbic acid, SH-groups, heteroauxin, starch, amino acids and to the substances of the coats were performed. The results obtained for the representatives of the *Compositae* and the Orchids are presented in the paper. A histochemical study of the peculiarities of the embryonic sphere in these plants gives an idea on very different pathways of the development that has lead these two families to their high position in remoted branches of the Angiosperm system.

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† Presented by Dr. E. A. Britikov.

Some representatives of *Taraxacum* genus (*T. officinale, T. hybernum, T. kok-saghyz*) were studied in the *Compositae*, while *Cypripedium insigne, Dendrobium nobile* and *Calanthe Veitchii* served as representatives of the *Orchidaceae*. These three Orchid species are taking different steps of the evolutionary development. The most primitive of them is *Cypripedium* genus, the most highly developed one – *Calanthe*, while *Dendrobium* takes the intermediate position. The development of the seed in *Taraxacum* is typical of the majority of highly developed *Dicotyledones*. Structural physiological adaptations intrinsic to them which increase the onflow of nutritive substances to the seed are highly developed and efficient in *Taraxacum*. Apart from the antipodes and integumental tapetum, accessory highly active haustorial-glandular structures appear in *Taraxacum* seeds.

The tissue of tapetum, adjacent to the tapetum itself, differentiates in the same direction as the cells of this latter. The tissue of tapetum shows intense reactions to proteins and oxidative enzymes, as tapetum does, which is a sign of its increased physiological activity. Along the margin of integument tissues, at the border with its hydrolysed zone, the secondary meristeme is formed which performs secretory functions clearly revealed in preparations treated with a reagent to ascorbic acid.

With the onset of the development of the embryo, the chalase in the *Taraxacum* ovule strongly increases in length, and protein-rich haustorial bands are formed in its cells contributing to the translocation of nutritive substances from the conducting system to the embryonic sac. At the base of the ovule, where a conducting bundle penetrates the seed, a group of cells differentiates which show intense reactions to proteins, ascorbic acid and peroxidase. This cell group also plays the role of the haustorial apparatus intensifying the onflow of nutritive substances from the plant to the seed.

The endosperm in *Taraxacum* develops normally during the early stages of the life of its seed but later on it is absorbed by the very active embryo. The *Taraxacum* embryo undergoes differentiation typical of the majority of the *Dicotyledones*: it develops cotyledones, the plumule and the root. The embryo shows distinct reactions to proteins, fats, amino acids, SH-groups, heteroauxin, and oxidative enzymes. These reactions demonstrate a high physiological activity of the embryo in *Taraxacum* and its richness in metabolites.

As a whole, the histochemical technique shows a high energy level in *Taraxacum* seed provided by a complex of haustorial-secretory adjustments. Their formation in *Taraxacum* genus can be regarded, as well as in other *Compositae*, as a continuation of the general development characterizing progressive evolution of the Angiosperms.

Quite a different picture is observed when histochemically studying

embryonic organs in the Orchids. Their ovules are completely lacking those structurally physiological adjustments which provide the ovules of the *Compositae* with a high level of physiological processes which is intrinsic to them. The embryo sac of the Orchids differs in the level of its physiological activity from that not only in the *Compositae* but also in the majority of other Angiosperms. The histochemical technique clearly reveals thereat the dependance of this level upon the position of a given species in the system. *Cypripedium*, as the most primitive Orchid, shows, from this aspect, less deviations from other Angiosperms than *Dendrobium* and *Calanthe*. In the embryonic sac of *Cypripedium* reaction to peroxidase is distinct prior to fertilization. In *Dendrobium* it is less clearly expressed, being completely absent in *Calanthe*. A weekly manifested activity of cytochromeoxidase and traces of heteroauxin are found in *Cypripedium*, being absent in *Dendrobium* and *Calanthe*. Reactions to SH-groups, amino acids, ascorbic acid are feebly manifested in *Cypripedium* and still more feebly in two other Orchids under study. The antipodal complex which plays in the Angiosperms the role of an energy apparatus stimulating the onflow of nutritive substances to the embryo sac is represented in *Cypripedium* by several cells still preserving haustorial activity. This can be judged by distinct reactions to ascorbic acid and oxidative enzymes. The antipodes of *Dendrobium* contain but traces of these substances, while the sole antipode of *Calanthe* does not participate at all in the stimulation of the onflow of substances through the chalase, since it transits from the chalasal to the micropylar zone of the embryo sac prior to fertilization.

The process of the morpho-physiological endosperm reduction can be followed in these same three Orchid species. In *Cypripedium* the endosperm rudiments are still preserved; 2–4 endosperm nuclei showing reaction to peroxidase and ascorbic acid are located just under the antipodes and seem to go on stimulating the onflow of substances from the plant to the embryo sac through the chalase. In *Dendrobium* the primary nucleus of the endosperm does not undergo division, while in *Calanthe* it is not formed as a rule.

Thus, the chalasal zone in the Orchid seeds gradually loses in the course of evolution all those haustorial structures which provide nutrition of the embryo sac in other Angiosperms.

How do the Orchids realize attraction of substances required for the embryo formation to their seeds?

An answer to this question can be found in the preparations of young *Calanthe* seeds treated with reagents to peroxidase. A strongly developed suspensor of the *Calanthe* embryo penetrating deeply into the micropyle shows an intense reaction to this enzyme which, as a rule, manifests a high activity in haustorial organs.

Thus, the suspensor haustorium replaces in the Orchids trophic mechanisms of the chalasal ovular region; it takes nutritive substances required by the embryo directly from the ovarial cavity filled with a solution which contains amino acids, reducing sugars and several other substances. The suspensor haustorium however, is unable to completely compensate the losses related in the Orchids with the absence of the endosperm, antipodes and other haustorial adjustments found in other Angiosperms. A result of the haustorial activity of the suspensor working alone is the formation of minute – measured in microns – seeds with tiny usually morphologically reduced embryos. Such embryos do not show reactions to oxidative enzymes and physiologically active substances (heteroauxin, sulfhydryl compounds). Their peculiar feature is that they do not show BIURET reaction although they are stained with bromphenol blue and show MILLONE reactions. This seems to indicate a peculiar structure of proteins which hides peptide bonds.

What is the cause of such a penetrating reduction of the Orchid seeds? There is no doubt whatsoever that their morphological reduction is underlain by physiological deficiency revealed histochemically and particularly striking when compared with the vigorous physiological equipment of the *Compositae* seeds. Then a question arises on the cause of this physiological deficiency. It seems to be the large number of the ovules formed in the Orchid ovaries. This number attains some dozens and hundreds of thousands, reaching several millions in some representatives. Due to the large number the ovules seem to suffer from the deficiency in both energy and plastic resources required for the construction of the organs upon which the whole further development of the seed depends. It should be noted, however, that Orchid ovaries themselves do not show any reduction typical of the seeds. The ovaries are rather large, they possess juicy chlorophyll-bearing tissues and a well developed conducting system. These tissues show intense reactions to oxidative enzymes and physiologically active substances. After fertilization the Orchid ovaries grow strongly which shows the ability of the seed to cause a strong stimulative effect upon them. This leads to the conclusion that, although the Orchid seeds do suffer from the trophical deficiency depressing the development of each seed separately, the sum-total physiological activity of the whole complex of seeds is sufficient to provide the onflow of nutritive substances to the ovary for its intense growth. It should be stressed that it is the ovaries in most highly developed forms characterized by especially small and multiple seeds which undergo the most intensive growth (in particular, *Calanthe*-ovary increases in size after fertilization much greater than that of *Cypripedium*). This shows that the sum-total phys-

iological activity of the Orchid seeds does not diminish but even augments in the process of evolution. Moreover, despite the ever more penetrating reduction of the Orchid seeds in the course of evolution, *Calanthe* seeds and those of other highly developed forms germinate, at least on a nutritive medium, much more easily and rapidly than those of more primitive Orchids, *Cypripedium*. This seems to indicate that, despite trophical deficiency suffered by the Orchid seeds, their physiological biochemical mechanism regulating germination continues improving in the course of evolution.

In other words, despite the reduction of seeds which seems to give to the embryonic processes in the Orchids rather a detrimental shade, physiological evolution of their generative sphere is of a progressive character and develops, although in a peculiar way, in the same progressive direction as the evolution of embryonic organs in the *Compositae*.

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AN ELECTRON MICROSCOPIC INVESTIGATION OF THE FILIFORM APPARATUS IN THE EMBRYO SAC OF *TORENIA FOURNIERI*

Introduction

Of about a hundred species of Angiosperms it is now certain that the embryo sacs in the micropylar part of the synergids contain a "filiform apparatus" (SCHNARF 1, VAZART 2). This structure was first observed by SCHACHT (3); the micropylar part of the synergids shows longitudinal striation and consists of a large number of thin threads. STRASBURGER (4) holds that the filiform apparatus is not striated but homogeneous and HABERMANN (5) discerns a honeycomb structure. By all probability, the filiform apparatus has longitudinal striation in the first stage of its development but later on becomes more or less homogeneous from the apex of the synergids downwards (SVENSSON 6, STEFFEN 7). There is no unanimity of opinion about the chemical composition of the filiform apparatus:

a. it consists of cellulose only (SCHACHT 8, HABERMANN 5, SVENSSON 6)
b. only the upper part consists of cellulose (STRASBURGER 4, VAZART 2)
c. it consists of cellulose and pectin (TISCHLER 9, ISHIKAWA 10).

CHIARUGI (quoted by SCHNARF 1) holds that the filiform apparatus consists of cellulose together with pectin. The quantity of pectin increases and reaches its maximum during fertilization and with this increase, the filiform apparatus becomes mucous. After the development of the egg apparatus has been completed, the filiform apparatus begins to take shape on the partition wall between the two synergids; here a strongly refractive, lenticular corpuscle develops which finally occupies the whole upper part of the synergids. Simultaneously with the development of the filiform apparatus the embryo sac wall above it is seen to grow thinner and thinner and to disappear finally (STRASBURGER 4). According to STEFFEN (7) the embryo sac wall is bulged forward in the micropyle by the growing filiform apparatus and then dissolves above it.

The filiform apparatus is supposed to play a part in the following processes:

a. the secretion of chemotropical substances

b. the penetration of the pollen tube.

The synergids produce chemotropical substances which leave the embryo sac through the filiform apparatus. The striae of the filiform apparatus are slender pores filled with protoplasm along which the substances to be secreted are transported (STRASBURGER 11). HABERMANN (5) thinks that the honeycomb structure of the filiform apparatus points to the presence of minute chambers through whose walls the secretion of chemotropical substances takes place. SCHNARF (1) remarks that the increase of pectin in the filiform apparatus up to the moment of fertilization suggests a similar function as was presumed by STRASBURGER (11). Apart from the secretion of chemotropical substances the filiform apparatus is supposed to have another function in the penetration of the pollen tube. SCHACHT (3) was the first to notice the relation between the presence of the filiform apparatus and the entrance of the pollen tube, but he was unable to indicate what exactly this relation consisted in. From SCHACHT onward, the discharge of the contents of the pollen tube into the embryo sac has been seen as follows:

a. the pollen tube penetrates through the embryo sac wall, bursts open and discharges its contents into one of the synergids (SCHNARF 1, SVENSSON 6, WYLIE 12, 13).

b. the pollen tube penetrates through the embryo sac wall and inside the embryo sac goes on to grow till it reaches the egg, where it empties itself (MADGE 14, STRASBURGER 4).

With *Impatiens glanduligera* the pollen tube eventually reaches the side or the apex of the filiform apparatus, travels through it while it becomes narrower but without opening, and does not eject its contents until it reaches the protoplasm of the synergids (STEFFEN 7). The synergid bursts open at the bottom and discharges the male gametes (SCHNARF 1, STEFFEN 7, SVENSSON 6, WYLIE 12, 13). In the lower part of the synergid, where the contents of the pollen tube have found their way, one or two densely staining particles, X-bodies, are found (COOPER 15, MAHESHWARI 16, WYLIE 12). Opinions about the nature of these X-bodies are very divergent.

The present investigation is concerned with the anatomy and the functional aspects of the filiform apparatus.

Material and Methods

Torenia fournieri has been chosen as the object of this investigation. The ovaries were cut open and fixed with veronal-acetate-buffered (pH 7,4) osmium tetroxide