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RECENT ADVANCES IN PHYSIOLOGY OF REPRODUCTION OF PLANTS[1]

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OUR knowledge of the physiology of sexual reproduction in plants is still rather limited and of quite recent origin. This is undoubtedly due to the fact that most higher plants are hermaphrodites and that the sexual organs are rather minute and ephemeral. It should be possible, however, to overcome this difficulty imposed by the material, at least in certain restricted phases of investigation of reproduction, by the use of large numbers of individuals, which permits the isolation of sufficient quantities of desirable tissues for a physiological and chemical assay. Then, too, the relatively small size and comparative ease of manipulation of many plants would seem to fit them to a variety of experimental observations and treatments.

For the purpose of orientation, sexual reproduction of higher plants may be subdivided into certain steps or phases, of which the following appear to be of major importance: (1) Initiation or "ripeness" of the sporophyte for reproduction; (2) chromosome conjugation (synapsis) and spore formation—the beginning of the gametophytic generation; (3) pollination and growth of the male gametophyte; (4) fertilization or union of gametes and (5) development of the embryo and its influence on the mother sporophyte.

For various reasons, which can not be taken into account here, some of these phases have been investigated more extensively or more thoroughly than others.

[1] Retiring president's address, American Society of Plant Physiologists, Atlantic City, December 29, 1936.

[2] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 506.

On not a few the experimental evidence is still very limited and of a conjectural nature.

Initiation of Sexual Reproduction

The genetic constitution of the plant determines not only the type of reproduction but to a large extent also the time of its occurrence. We have plants of typical floral organs and late and early blooming varieties and strains in great abundance. Ordinarily these features are quite specific and stable. Consequently, it was believed for a long time that environmental factors, though effective in modifying the action of genes, were not able to delay or alter greatly the fundamental developmental sequence in plants. It was supposed to be entirely under genotypic control.

The striking results obtained by Klebs at the turn of the century showed, however, that a prolonged exposure to certain external factors, mainly light and temperature, may result in either continuous vegetative growth of plants or, contrariwise, they may be induced to become reproductive prematurely. Thereby the normal developmental sequence could be altered most markedly. Plants could be stopped and maintained at particular stages of development almost indefinitely or made to proceed through a series of stages with astonishing rapidity. According to Klebs, environmental factors have no direct effect on the protoplasm. Their action is expressed through changes in the internal environment of the plant, which in turn has a bearing on the function of the living part of the organism. He posited as the primary cause the accumulation of carbohydrates and their relative concentration in comparison to soil nutrients, especially nitrogen. During the vegetative state the plant is characterized by a relatively low concentration of carbohydrates and in the reproductive state by a high accumulation of the products of photosynthesis.

The concept of the carbohydrate-soil nutrient relationship as a factor in the initiation of reproduction in plants remained dormant for almost two decades. Possibly it came at the "wrong" time, since the biologists were then interested in the new theory of insulation of the germplasm from its environment, which was promulgated by Weismann and accepted readily.

The ideas expressed by Klebs were taken up some years later by Kraus and Kraybill, who tried to establish more definitely the carbohydrate-nitrogen relationship as a causal factor in both vegetative development and sexual reproduction of plants. It is not necessary to detail here the results of investigations supporting this idea and those contradicting it, since all students interested in plant physiology are quite familiar with them. The prevailing opinion at present seems to be that, though the carbohydrate-nitrogen ratio may have something to do with the amount of reproductive organs formed or the quantity of fruit produced, it is not the causal agent of initiation of the reproductive state. There remains the possibility, that a certain carbohydrate-nitrogen environment may be conducive to the production of a specific catalytic substance, which initiates reproduction.

That environmental factors have a striking, almost direct effect in bringing about sexual reproduction in higher plants was demonstrated by Garner and Allard through their studies of photoperiodism. No one would have suspected that so "dilute" a part of the environment as length of day would have so potent an effect on development of plants.

Studies of the nature of photoperiodism have been influenced by the fact that the response can be localized. It is not yet certain, however, whether reception of the photoperiod is restricted to the stem or to the leaves, though most of the evidence seems to point to the leaves, whence the effect is transmitted to the stem. It has been suggested that the chloroplast pigments, especially the carotinoids, may have something to do with it and that the oxidation-reduction enzymes may determine the reaction of plants to length of day.

Several investigators have demonstrated that when certain plants are grown for a definite time under one photoperiod and then transferred to another, the effect of the first exposure will appear in later development. This phenomenon has been named "photoperiodic after-effect" or "photoperiodic induction." Thus by exposure to an appropriate length of day sexual reproduction may be induced and will manifest itself, though the plant may be grown subsequently in an environment conducive only to vegetative growth. Instances are on record where as few as 4 to 6 days have been sufficient for induction of reproduction.

The causal mechanism of photoperiodic induction as that of photoperiodism itself is unknown. It is possible that irreversible changes are brought about during the period of induction in the protoplasm of the growing points, which speeds up the development of the plant. There is clear-cut evidence that a certain "dosage" or number of days are required to induce *fully* the initiation and growth of the reproductive organs. Treatments short of this necessary minimum will result in incomplete or partly vegetative flowers. The mechanism of photoperiodism, therefore, is not "trigger-like" in its action, as has been suggested. Very likely it exerts itself through the formation of certain hormone-like substances, the quantitative accumulation of which results eventually in the development of reproductive organs.

Much activity has been displayed during the past few years in studies of the relation of temperature to photoperiodism and more lately on the direct effects of temperature on sexual reproduction of plants. Of greatest interest at present is not the fact that temperature may modify the formative effects of the photoperiod, but that this environmental factor itself may be as potent in induction of reproduction as the light period. The extensive investigations by Thompson and his coworkers show that as small differences as 6–10° F. will either suppress or stimulate the formation and development of floral organs.

Apparently the length of day is not the only external factor that may cause the initiation of sexual reproduction. Plants seem to be more sensitive to environmental conditions than we have suspected. It is probable, therefore, that there may be other external agencies yet to be discovered, which, at least under certain circumstances and with particular groups of plants, may be as effective as photoperiods and temperature in their influence on development.

A quite unsuspected approach to physiology of reproduction of plants has been made through studies of *vernalization*—a treatment given to seeds previous to sowing to hasten the time of flowering. This procedure, though suggested by Gassner, was initiated by Lysenko. By exposing partly swollen seeds to definite temperature, depending on the species, an induction of reproduction is obtained, which is very similar to photoperiodic induction or after-effect. This treatment itself, however, will not make plants reproductive unless during their subsequent development they are exposed to a suitable length of day. Many plants, therefore, have to pass through two, possibly more, stages or phases of internal readjustment—the thermo- and the photo-phase at least—before they become sexually reproductive.

The extensive investigations on vernalization and speculation as to the essential nature of the response of plants to this treatment has led Lysenko to a conception known as *"phasic development."* Though this author postulates a strict succession in development, it would seem that in *Soja* and possibly other species vernalization implies the completion of both phases (thermo and photo) more or less concurrently. Of significance in this connection is the suggestion that it may be possible to vernalize by light only and that some seeds may become vernalized while still connected with the mother sporophyte.

From the results of recent studies the striking fact emerges that it is possible to initiate reproduction in a very early stage in the development of the plant—while it is in the seed. Hence some phases of the physiological studies of sexual reproduction, perforce, will have to be moved back to the seed stage of the plant.

Since no flower primordia are present in this extremely early period of development, the after-effect from vernalization, and most probably from photoperiod also, must express itself through alteration in the physiological state of the whole or some parts of the embryo plant. This would seem to suggest a chemical substance, possibly of catalytic nature, as the causal factor bringing about these results.

Changes produced in cells of plants in the embryonic stage as a result of vernalization seem to be transferred to other cells produced from them, but not translocated to other parts of the same plant. This appears to be true for both the thermo- and the photophase.

Vernalization during the thermo-stage may be given in instalments as it were: half of the total exposure now, then seeds dried, and the other half later. The results from the treatment are additive. Hence the influence exerted by temperature appears to be of the nature of gradual quantitative effects. Recent evidence suggests that the effects from a photoperiod are also of a "quantitative" nature and that a time factor exists for the accentuation of the induced changes. By a careful regulation of the period of exposure to certain lengths of day, one may obtain various types of development between vegetative shoots and normal flowers. This, again, seems to point to a chemical hormone-like substance as the causal agent.

Once reproduction is initiated, it proceeds through more or less definite morphogenetic stages—synapsis and the formation of spores, gametogenesis, pollination, fertilization, embryo development and its ripening into a seed. Naturally the mother sporophyte participates in, is part of and is affected by the special morphological development and physiological changes during the reproductive cycle.

SYNAPTIC OR CHROMOSOME CONJUGATION STAGE

This very important phase in the alternation of generations of plants is not reserved solely for the study of geneticists, though they have developed a very profound technique and a respectable vocabulary for the analysis and interpretation of certain very important features of it. If during gametic union or fertilization there is a physiological stimulation, which results in rapid development of tissues accessory to the embryo and may stimulate other parts of the plant, then one should expect also a physiological effect during chromosome pairing or conjugation at synapsis, which is supposed to be the final stage or completion of fertilization. This idea was expressed by the speaker some time ago, though the experimental data to support it were limited.

A spurt in growth of various parts of the plant in proximity to the developing flower buds is a familiar phenomenon. It is somewhat difficult to say wherein lies the exact cause of this stimulation. Circumstantial evidence seems to point to the synaptic stage of reproduction as the period of initiation of this acceleration in growth. Vöchting and Fitting have studied experimentally the relationship between the flower bud and growth of adjoining tissues. More recently Katunsky has analyzed the problem histologically and has pointed out the existence of a correlation between the development of primary reproductive tissues and the accessory organs. In *Papaver* and *Crepis,* for example, the stages in growth and movement of the pedicels correspond with definite phases of the development of the female gametophyte. Two maxima could be observed. The first occurred during the development of the nucellus, the second and larger spurt in growth took place at the approximate time of reduction division and spore formation. Rate of cell division and growth in both instances is thought to be due to the production of a growth-promoting hormone by the pistil, more specifically by the developing ovule.

Pollination and Growth of the Micro-gametophyte

The germination of pollen grains and growth of pollen tubes seems to be controlled not only by environmental factors and the nutrient supply of the stigma and style but also by specific accessory substances. These may be either catalytic or inhibitory in nature or both. To what extent the production of these regulators is subject to the genic constitution of the plant and how much it is determined by the internal physiological environment is still an open question. That the growth of the pollen tubes through the style has a stimulating effect on the gynoecium has been known for a considerable time. In the absence of fertilization, the fruit frequently develops parthenocarpically as a result of this stimulation.

A more concrete demonstration of this effect has been supplied by Gustafson, who has been able to induce parthenocarpy by pollen extracts and, what is more interesting, by the application of growth-promoting chemicals (indole and phenyl derivatives) to the cut surface of the style just above the ovary. This makes it quite evident that definite substances are released by either the microgametes or the microgametophytes. These do not seem to be specific and may be related to the auxins. However, one is obliged to view with reservation a too wide application of the few plant hormones known at present.

Of interest in this connection are the investigations by Fitting, who has demonstrated that in some plants, especially orchids, the petals and other floral parts undergo senescence and death soon after pollination and long before fertilization has occurred. In the absence of pollination the flowers will not die for some time. The wilting of flowers thus seems to be due to the secretion of certain substances by the pollen. Such an effect is obtained also when pollen of a species incapable of accomplishing fertilization is applied to the stigma.

It is possible that in some instances the presence of the male gametophyte in the style results in parthenogenesis—the development of the embryo without fertilization. The conspicuous results obtained by artificial (chemical) fertilization of eggs in animals suggests such a possibility.

Fertilization and Formation of the Zygote

There is considerable experimental evidence at hand showing the influence of fertilization on the physiology and morphology of plants. It is commonly known that normally the accessory tissues making up the fruit are able to develop only in the presence of one or more embryos. If fertilization has not taken place and the zygote is not formed, the fruit will grow but little and will absciss while still immature. Exceptions to this rule, of course, are cases of parthenocarpy, referred to already.

As a result of the act of gametic union, and hence immediately following fertilization, there is a marked increase in metabolism in tissues adjoining the embryos. It is not known as yet whether nuclear division of the endosperm is also stimulated by the presence of the embryo. This 3n generation may have its own high metabolic gradient. The nucellus and the integuments are certainly affected by the embryo, in the absence of which they usually do not develop and undergo atrophy a few days after pollination (*Pyrus,* etc.).

Of interest in this connection is the possible fact that in *Citrus, Mangifera* and other species, the presence of the embryo may lead to proliferation of nucellar tissue resulting in production of apogamic embryos. It is not clear, however, whether apogamy is caused by stimulation produced by the embryo or, somewhat earlier, by the macrogametophyte.

Stimulation from the embryo naturally extends to ovarian and other tissues making up the fruit, which will be either large or small, regular or irregular in form, and altered in chemical composition, depending on the number, size and position of embryos present. These well-known facts to the botanist and horticulturist have been submitted recently to experimental verification by Tukey, who has demonstrated in various ways that the development of stone fruits is affected by the embryo. In this relationship between the seed and the pericarp the stage of development of the embryo seems to be one of the main factors.

In discussing the effects of fertilization on growth of tissues of the mother sporophyte, one should not lose sight of an important genetic aspect. The increased or decreased heterozygosis, resulting from certain pollinations, frequently has an observable and measurable influence on the size and composition of the pericarp and accessory tissues of the fruit. This has been demonstrated with the date, cotton, legumes and several other plants. The phenomenon has been named *metaxenia,* thus separating it from xenia, the visible genetic influence on the endosperm of monocots or cotyledons of dicots.

In many plants (*Tussilago, Digitalis, Althea*) the greatest growth of the flower stem occurs after fertilization. It ceases when cell division of the embryo is completed. In some cases fertilization is essential for the growth and movement of the pedicels and other floral parts. Effects obtained from pollination do not seem to bring about these changes.

Stimulation due to sexual reproduction seems to extend for some distance beyond the reproductive organs and probably throughout the organism. The speaker has found that in the tomato (*L. esculentum*) the development of embryos and accessory tissues influences the whole metabolism of the plant. As a result of gametic union and formation zygotes, both absorption of soil nutrients and assimilation of carbon dioxide are increased. It is possible to measure this effect only when the number of embryos per plant is quite large and the fruit is removed shortly after fertilization. This procedure prevents the masking of the effect by changes brought about by the "metabolic drain" or monopolization of various substances by the rapidly developing fruit.

Though a general stimulation may accrue from fertilization, it is quite clear that the fruits obtain an incomparably greater profit from it. The rest of the mother sporophyte receives but a temporary exhilaration, as it were, the benefits of which go eventually and often entirely to the developing seeds and fruits. Once the largest number of embryos have been formed under the existing conditions of nutrition, almost all the synthesized and a large part of the directly absorbed substance are incorporated into the fruits—the organs directly associated with the life of the embryos.

What are the means by which the embryos are able to draw to themselves and adjoining tissues the available food supply? In light of the present experimental evidence one is obliged to postulate a mechanism of a specific enzyme or hormone character. At certain stages of growth the embryos may secrete a hormone or hormones, which may regulate metabolism and direct development. The suggestion of an endogenous system of secretions is in line with the newer knowledge of plant physiology.

EFFECTS OF EMBRYO DEVELOPMENT ON MOTHER SPOROPHYTES

One of the most striking features of physiology of reproduction of higher plants is the diversion or localization by the embryo of the products of assimilation. As a result of this draft on the available food resources of the plant all sorts of inhibitions, disarrangements and even destruction of certain parts of the parent organism may take place. In species with a continuous type of growth, like the tomato, a maximum crop of fruit results in curtailment of growth and development in approximately the following order: (1) Sterility of blossoms; (2) decrease in size of flowers and the inflorescence; (3) abortion of flower buds; (4) reduction and cessation of terminal growth of stems and branches; and eventually (5) complete exhaustion and death of the plant, save the fruit.

Since the number of seeds and fruits formed does not always overtax the capacity of the plant to support, there may not be a very noticeable effect or only a temporary curtailment in growth and development or in flowering and fruiting. As soon as the fruit crop has absorbed all the required nutrients, growth is resumed. This may cause cyclic development and fruiting, which is exhibited by many groups of plants. Alternate bearing of fruit trees, periodic flowering, cyclic sterility, etc., are phenomena that may be traced back to the effects of crops of seeds and fruits on the metabolism of the plant.

It is not known for certain what particular substances may be exhausted first by the reproductive organs and thus act as limiting factors. There are several reasons to believe that frequently nitrogen may be the first limiting factor: (1) Seeds and young fruits are organs of relatively high nitrogen content. (2) Nitrogen frequently runs short in rapidly growing plants. (3) A crop of fruits inhibits growth and changes development in a manner that is symptomatic of nitrogen starvation. (4) Plants of low nitrogen content are affected sooner than those abundantly supplied with this element. (5) Ample nitrogen nutrition will delay the "detrimental" effects until additional fruits are formed and the "load" has reached again the capacity of the plant to support.

Naturally other organic substances, especially the carbohydrates, under certain conditions may be the limiting factors. While during the early stages of growth the fruit absorbs much nitrogen, later on it accumulates mostly carbohydrates.

The speaker is fully aware of the fact that he has attempted to cover a very broad and still little studied subject of plant physiology, that he has neglected entirely the lower plants and that he has omitted certain phases of physiology of reproduction of the spermatophytes. The time and his own interests have to some extent limited this discussion. Let us hope that in the future more attention will be paid to this phase of the life of plants, that in summarizing our knowledge of plant physiology there will be a substantial fund of more exact information on the physiology of reproduction of higher plants.