Experimental Study of Floral Morphogenesis

II. Contribution to the Morphology of Inflorescence in *Zea mays* L.

Experimentální studie květní morfogenese
II. Příspěvek k morfologii květenství *Zea mays* L.

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A b s t r a c t — A maleic hydrazide solution, properly applied, is capable of disturbing the normal succession of floral primordia differentiation in maize, calling forth a series of abnormities, which manifest metamorphosis of floral parts and enable to comprehend some of the morphological peculiarities in the structure of the tassel and the ear. The induced abnormities corroborate the existence of potential hermaphrodite character of the florets in both inflorescences, as well as homology of the ear rachis with the central axis of the tassel. Acropetal succession in the development of floral primordia enables to disturb the normal course of development in different stages and thus to bring about abnormities of different quality. An intervention in the development of floral primordia in the more advanced stages, at the ear base, results of floral primordia in the more advanced stages, at the ear base, results in the origination of flowers with gynoecium containing two or three ovules. From these gynoecia there develop, in sporadic cases, kernels (grains) with two embryos. Stigmas and styles divided at varying depth show that a normal style must have originated through coalescence of two styles. The next free style grows out of the protrusion under the stylar canal. An intervention in the undifferentiated spiklet primordia in the middle of the ear has for its result the origination of several free gynoecia. If we however, interfere with the development of altogether undifferentiated parts of the ear, we bring about branching of the maize cob and the initiating of staminate, hermaphrodite or pistillate flowers. The occurrence of an actinomorphic hermaphrodite flower with one lemma and two paleas, three lodicules, and six stamens points to a connection with the hypothetic floral diagram of the *Poaceae*. The quoted examples show that the reproductive meristems recapitulate, in the course of ontogeny, several stages of phylogeny. An intervention in the quite early stages of ontogeny calls forth fundamental changes, which evidently result in the occurrence of the very primitive monocotyledonoid types. An intervention in the more advanced stages of the primordia development gives rise to abnormities of lower ranks, i.e. with characteristics of the families, genera, and species, respectively.

The morphological structure of the maize inflorescence differs from the other members of the grass family by displaying a number of peculiarities, whose investigation is not only significant from the practical point of view, but may also contribute considerably to our theoretical knowledge. One of the most outstanding features of *Zea mays* L. is its separate (unisexual) inflorescence.

The terminal staminate tassel is composed of a central axis with numerous pairs of spikelets in a spiral arrangement and of lateral branches bearing paired spikelets in two rows only. Each pair emerges from a common base and consists of one pedicellate terminal, and one sessile lateral spikelets. Each spikelet is composed of long glumes and two staminate flowers (florets). A floret consists of an awnless lemma, an equally long palea, two lodicules, three stamens, and a rudimentary pistil.

The lateral pistillate inflorescence forms a symmetrical ear with a considerable number of spikelets pairs attached to it in longitudinal rows. The spikelets grow on such short pedicles

(rachillas) that it is hard to distinquish the pedicellate or sessile spikelets from one another. In spikelets with two short, thick glumes only the upper pistillate floret develops. The lower one gets usually soon aborted, leaving behind it, in the maturity, only a distinct lemma, which used to be mistaken by former taxonomers for a third glume (HEGI 1906). A fertile flower contains a fully developed pistil, tree aborted stamens, and two indistinct lodicules. The pistil consists of a round with one ovule and two integuments, the same having a stylar canal and a tiny protuberance below it on the ovary wall. The short style finds its continuation in a long stigma with a biparted tip. The flower is enclosed by a short, thin lemma and a palea. The entire pistillate inflorescence is enveloped in modified leaves with reduced blades.

The homology of the ear and the tassel was the subject of numerous studies. It was found that the ear is a paniculate inflorescence, whose many parts, in the course of evolution, have been subjected to a reduction. The massive corn cob originated through vertical condensation of the primary inflorescence axis and by an adnation of the prophylls of spikelet branches to it (NICKERSON 1954). Formerly the origin of the ear from the tassel was explained by pointing to the fusion of lateral branches with the central rachis of the tassel (VELENOVSKÝ 1910, COLINS 1919, BURDICK 1951). It was not until the spiral arrangement of the spikelet pairs on the ear rachis was correctly understood (FUJITA 1939) that the research workers began to consider the ear rachis homologous with the central axis of the tassel only. STEPHENS (1948) saw in every pair of the ear spikelets a strongly reduced lateral branch of the tassel emerging from the axil of a reduced bract. He demonstrated his standpoint by giving an analysis of vascular anatomy in the nodal system of spikelets lying in a cupule, formed by an adnate prophyll and a ridge of suppressed bract.

As for the homology of floral parts of the maize, views still greatly differ; no wonder since even the homology of floral structure of the grasses, which seems to form a contrast to the trimerous type of flowers in the monocotyledons, lacks clarification. Morphologists took formerly the lemma and the palea for the outer perianth while the lodicules for the inner periant of the originally dimerous flowers (SCHENCK 1890).Other morphologists deny the existence of the perianth in the grass flower altogether, seeing in the lemma the bract and in the palea the bracteole (NICKERSON 1953). A quite different concept was offered by ČELAKOVSKÝ (1895), who tried to derive floral structure from the more primitive forms of tropical grasses. According to this view, we find in the axil of the bract pentacyclic, syncarpous, trimerous flowers with alternate whorls. From the outer trimerous whorl the median sepal had disappeared, while the remaining two became adnate, forming the palea. As to the inner whorl of the perianth the median petal is usually suppressed and the remaining two turn into lodicules. In the original flower there existed six stamens arranged in two alternate trimerous whorls. In most of the grasses the inner whorl aborted, and the outer whorl was all that was left. The pistil, which was tricarpellate by origin, appears to be an indivisible unit, not bearing, as a rule, any traces of carpel coalescence.

DEYL (1955) doubted the correctness of this interpretation, which makes of the reduction processes in the floral structure the main evolutionary principle, ascribing little credibility to the origin of the unstable grass family from the stabilized condition characterizing the *Liliaceae*. He prefers to start from HAECKEL's concept maintaining that the flower of grasses has no perianth, and he takes the lemma and the palea for bracts and the lodicules for small bracts. Their distichous arrangement and considerable variability (splitting or abortion) explains better, according to DEYL, the variable character of the leaf than of the far more stabilized flower parts. He believes the gynoecium of the *Poaceae* to be an one- carpel gynoecium because it gives rise to the caryopsis, which may originate in a similar way as the achene of *Typha* from the one-carpel ovary only. He finds corroboration of his theory in the discovery of more caryopses in flowers of *Triticum vulgare* hybridized with *Elymus arenarius* (CICIN 1954).

The above mentioned differences in the standpoints of comparative morphologists and taxonomers concerning the origin of floral parts may be bridged only by experiment and by analyzing concrete instances. It becomes clear that a suitable metod for this sort of study can be based on developmental anatomy (BONNET 1953) and experimental morphology. DOSTÁL (1959, 1960) showed that the apical meristems recapitulate in the beginning of the ontogenetic development some stages of phylogeny. Deliberately inflicted disturbance of the normal succession of differentiation of the apical meristems results, as a rule, in the rise of ancestral forms, which betray several traces of phylogenetic relationships between genera and families, and may contribute towards the solution of evolutionary peculiarities. In our previous paper (SLADKÝ 1966a) we have shown how maleic

hydrazide, suitably applied, can disturb the normal succession of floral primordia differentiation in *Campanula rapunculoides* L. and in *Veronica austriaca* L. subsp. *austriaca*, calling forth a number of abnormities. These studies made it spossible to follow the laws of metamorphosis of floral parts and to observe the phylogenetic affinities. With the application of similar methods we have approached also the above mentioned problems concerning the floral structure of the two inflorescences of *Zea mays* L.

Material and Metod

For our experiments we have used young maize plants (single cross WD × W9), which were grown from May to July 1964–1966 in experimental beds in normal agrotechnical conditinns. We sprayed the plants before the start of differentiation of floral primordia, as well as in its course, with a 0,1% solution of maleic hydrazide, directing the spray on the leaves, this being done twice each time with one week's interval. The acropetal succession of development in both racemose inflorescences enabled to disturb the differentiation process of floral primordia in most various stages of development and growth. The floral abnormities were evaluated with respect to their position in the inflorescence, the object being to compare the intervention in a certain stage of development of the floral and spikelet primordia with the character of the arising abnormity. The course of floral differentiation was followed in a microscope, and some stages were photographed.

Results of experiments

The initial stages of the tassel and ear differentiation are in *Zea mays* L. morphologically identical. Plate I reproduces the common stages of this development. The vegetative shoot apex (Fig. 1) gets considerably elongated and enlarged in the process of its change into the floral meristem (Fig. 2). Through the cell division of the tunica layer there originate pairs of spikelet primordia in a spiral arrangement (Fig. 3), and in each of these primordia the upper and the lower flower gets differentiated. Differentiation of the spikelets proceeds in both inflorescences in acropetal succesion (Fig. 4). The differences between the tassel and the ear are not perceivable until the lateral branches of the tassel begin to grow (Fig. 5b) and the flower differentiation becomes evident in the spikelets. While in the terminal tassel both floral primordia get differentiated and form staminate flowers, in the ear spikelets only the upper flowers develop. In them we can observe the diferentiation of the gynoecium with the style and stigma. The ear rachis expands in the course of further differentiation (Fig. 6).

Plate II depicts the transformation of the staminate tassel primordia into the pistillate ear. In each tassel floral primordium there originates besides the stamens the pistil primordium (Fig. 1 an, pi). A mild inhibition interference effected by maleic hydrazide and affecting the differentiation course in the tassel primordium results in a feminization of the flowers in spikelets on the main axis and also in the suppression of growth in basal branches of the tassel. In accordance with the degree of development in the floral parts there originate either hermaphrodite flowers or only pistillate ones (Fig. 2 pi). The feminization of the spikelets is usually accompanied with an accelerated growth of the upper flower with the style (Fig. 3 si). The growth of the lower flower is inhibited and finally it gets altogether aborted. The glumes, lemmas and paleas resemble in their structure analogous floral parts of spikelets in a normal ear. When the right moment in the differentiation is chosen for the application of maleic hydrazide, we can obtain a purely pistillate inflorescence. If the inflorescence contains, already prior

to the intervention, primordia of basal lateral branches, there arises from the primordium of the central axis of the tassel a pistillate ear, and at its base, from the spikelet pairs, we can observe the development of lateral branches with staminate and pistillate flowers (Fig. 4b). A double spray with maleic hydrazide ensures the origination of a typical terminal ear, which is sheathet in leaves of the main stem.

The differentiation in the ear spikelets is slower than that of the tassel ones, being characterized with a number of reduction processes. Plate III reproduces the anatomical and morphological structure of the normal pistillate spikelet along with abnormal spikelets which came into being after an intervention in the more advanced stages of the floral primordia differentiation. As we have already pointed out, a normal spikelet (Fig. 1) of the ear contains both the adaxial and the abaxial glume (g) and the primordia of two flowers (fl_1, fl_2). Yet, it is only the pistillate flower with its lemma and palea which develops (le, pa). The stamen primordia get aborted in the early stages of development. The round gynoecium contains one large anatropous, dichlamydeous ovule (ov). The short style finds its continuation in a long stigma a biparted tip (si). The primordia of all parts of the lower flower (fl_2) gradually undergo a reduction with the exception of the lemma, which persists.

The acropetal succession in the development of the ear spikelets enables to disturb the developmental course in its different stages and thus to call forth abnormities of different quality. An intervention in the more advanced stages of differentiation of floral primordia, at the base of the ear, brings about changes which resemble the characters of the more primitive species, genera, and families. The most frequent occurrence is the growth of the lower flower (Fig 2 fl_2), so that in the spikelets we can observe the development of two pistils (Fig. 3 pi_1, pi_2). The pistil of the upper flower develops, as a rule, quicker, but if the concentration of maleic hydrazide is stronger or the intervention earlier, we may face the opposite situation. In some flowers, the stamens are for the most part preserved in a reduced condition (Fig. 3 an). In the spikelets nearer to the center, we sometimes find even three separate flowers with pistils (Fig. 4 pi_1, pi_2, pi_3).

In the next plate IV further types of abnormities are presented, which originated after interventions in various stages of differentiation of the floral and spikelet primordia. At the base of the ear, gynoecia appear with two unequal thick styles and stigmas (Fig. 1 si). The upper, larger style grows out of the front ovary wall and has a normal structure with two vascular bundles and a biparted tip. The division of the stigmas is not always of the same length and often affects also style. The other, more narrow style with a stigma grows out of the protuberance in the ovary wall under the stylar canal, and contains one vascular bundle. The origin of a free style shows that the slight protuberance in the wall of a normal gynoecium is, in fact, the reduced third style, just as it is the case of *Oryza sativa* L. The gynoecia of these abnormal flowers contain often two or three ovules (Fig. 2 ov). The ovules are smaller, but also anatropous and dichlamydeous (i). The occurrence of three ovules and the development of another free style with a stigma speak in favour of three-carpel origin of the gynoecium. When the ears of experimental plants were harvested, we could observe some kernels germinating directly on the ear. In four cases we found kernels,

each of which had two plumules and two primary roots and a single coty-ledon.

An intervention in the undifferentiated spikelet primordia in the middle of the ear results in the origin of several free apocarpous pistils in the flower (Fig. 3 pi). Each gynoecium contained one small anatropous ovule, and ana-logically the style had only one vascular bundle. In other cases gynoecia coalesced, but the styles with stigmas kept separate. From these gynoecia no kernels originated.

In the apical part of the ear the effect of maleic hydrazide results mostly in braching of the rachis. The branches growing from the axil of spikelet glumes resemble lateral branches of the tassel and bear along with the pistil-late flowers also staminate flowers. In the transition zone between the pistil-late and staminate flowers one actinomorphis hermaphrodite flower was found (Fig. 4), having between the lemma and the paleas three lodicules (lo), six stamens (an), and one pistil (pi). The structure of this flower closely resembles the original floral diagram of the *Poaceae*. This ancestral flower indicates that a considerable simplification of the floral structure must have taken place in the course of phylogeny, and corroborates the significant role the inhibitive regulation plays in normal processes of differentiation.

Discussion

From the literature (LANG 1965) we know, that the course of morphoge-nesis, as well as the final differentiation of the flower, are greatly influenced by the level of endogenous auxins in the meristems. HESLOP-HARRISON (1961) points out that an increase of auxin level in the plant brings about, on the one hand, suppression of the sepals and androecium, and, on the other hand, it promotes hypertrophy of sepals and gynoecium. SLADKÝ (1966b) stated that even the differentiation process of the tassel and the ear in *Zea mays* L. is associated with a different level of auxin in apical and lateral meristems. An increase of the auxin level in a tassel primordium results, during the further development, in its transformation into a pistillate ear. A suitable application of maleic hydrazide increases natural level of auxin in the terminal primordia of the tassel, which promotes feminization of the flowers.

A double spray with maleic hydrazide is necessary, because the regulation system in the plant tends to inhibit the effect of a single spray, while the concentration of maleic hydrazide in a spray cannot by increased, unless it should be altogether inhibitive. The fact that disturbance of the early stages in the spikelet primordia development produces the more primitive forms, whereas an intervention in the more advanced stages of floral pri-mordia gives rise to more derived forms shows that the meristems of shoot apices recapitulate briefly in the beginning of their ontogeny some phylo-genetic stages. The results point to a close and delicate relation between ontogeny and phylogeny, expressed in 1866 by HAECKEL in his biogenetic law. In spite of skepticism of some taxonomers (WETTSTEIN 1933, DE BEER 1951) the validity of the biogenetic law finds support in the comparative studies of ZIMMERMANN (1930, 1950) and in the recapitulation experiments of DOSTÁL (1959, 1960). Analogically the results of our former paper (SLADKÝ 1966a) and of the present study tend to substantiate the validity of the biogenetic law.

Interpretation of the results in this work enables to explain the diversity of views concerning the origin of the grass gynoecium. DEYLS (1955) concept, as to the formation of several caryopses in flowers of hybrids *Triticum* × *Elymus*, may be explained by the origin of several separate apocarpous gynoecia that are in accordance with the more primitive families. Essentially they resemble our abnormities (plate IV, Fig. 3), and their appearence is probably the result of a genetival disturbance at the very beginning of floral initiation of the spikelets. The occurrence of the more derived forms, of the gynoecia with three ovules and three styles, seems to point more to the three-carpel character of gynoecium, as ČELAKOVSKÝ assumed, than to just a restricted reduction of the number of originating from one carpel. This type of abnormity arises from more advanced primordia, i.e. in flowers at the ear base.

The occurrence of an actinomorphic hermaphrodite flower with six stamens, three lodicules in the apical part of the ear may be considered as a perpetuation of the stimulation effect of maleic hydrazide, which affected the early stage of differentiation of inflorecence itself. And here again we encounter a remarkable conformity between this type of flower and the hypothetically assumed flower structure of the *Poaceae*.

In the literature (SPRAGUE 1955), the origination of two or more embryos in one kernel is interpreted as manifestation of polyembryony. KIESSELBACH (1926) calls, in fact, this phenomenon false polyembryony, believing that here we have to deal with two embryos originating through splitting of one embryo. Other authors (WEATHERWAX 1935) try to find the cause in cytological abnormalities of the mother cell or in the cells of the embryo sack. As a rule the three-carpel origin of gynoecium is being overlooked and the same is done with the recapitulation processe during ontogeny. Or study shows that in the gynoecium there originate two or three ovules, from which mature embryos with endosperm in a common pericarp of the kernels may develop. LUXOVÁ et LUX (1964), and ERDELSKÁ (1966) demonstrate the origination of two or more ovules in the flowers of *Hordeum sativum* L. after the application of MCPA in early stages of development.

From the above mentioned examples we may conclude that experimental inducement of abnormities enables to follow processes which, to a certain extent, elucidate the metamorphosis of floral parts and contribute towards the solution of the problems of evolution.

Souhrn

Maleinhydrazid při vhodné aplikaci naruší normální sled diferenciace květních základů kukuřice a vyvolá škálu abnormit, které prozrazují metamorfózu květních částí a umožňují pochopení některých morfologických zvláštností laty a palice. Experimentálně vyvolané abnormity potvrzují potenciální obupohlavnost květů obou květenství a homologii vřetene palice s centrálním vřetenem laty. Akropetální posloupnost vývoje květních primordií umožňuje porušit normální průběh vývoje v různých etapách a vyvolat abnormity různé kvality. Zásah do vývoje pokročilejších květních základů na bázi palice vede ke vzniku květů s gynaeceem obsahujícím dvě nebo tři vajíčka. Z těchto semeníků v ojedinělých případech vznikají obilky se dvěma zárodky. Různě hluboko rozdělené blizny a čnělky ukazují, že normální čnělka vznikla srůstem dvou čnělek. Další volná čnělka vyrůstá z výběžku pod čnělkovým kanálkem. Zásah navozený do nediferencovaných primordií klásků ve středu palice vede ke vzniku několika volných gynaeceí.

Zásah do vývoje zcela nediferencovaných apikálních částí palice vyvolá větvení vřetene a vznik prašníkových, oboupohlavných nebo pestíkových květů. Výskyt aktinomorfního oboupohlavného květu s pluchou a dvěma pluškami, třemi lodikulami a šesti tyčinkami ukazuje souvislost s hypotetickým květním diagramem čeledi *Poaceae*. Uvedené příklady ukazují, že pletiva reprodukčních meristémů rekapitulují během ontogeneze některé etapy fylogeneze. Zásah do nejranějších fází ontogeneze vyvolá změny základní, při čemž se objevují zřejmě prapůvodní typy monokotyledonoidní. Zásah do pokročilejších etap vývoje vyvolá abnormity se znaky čeledí rodů a druhů.

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See also plates XV—XVIII in the appendix.
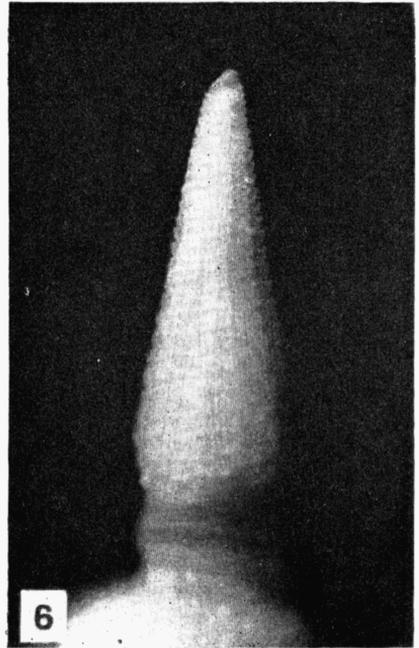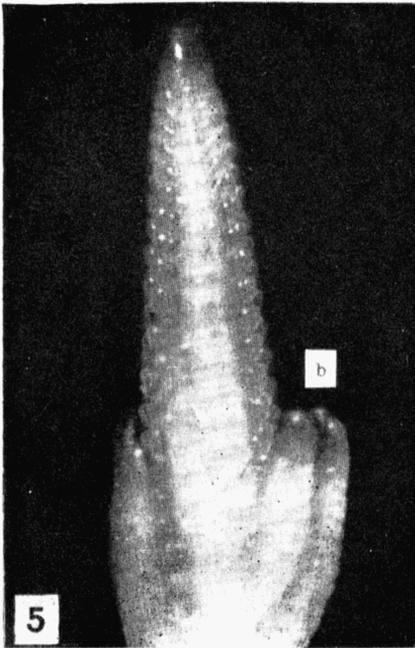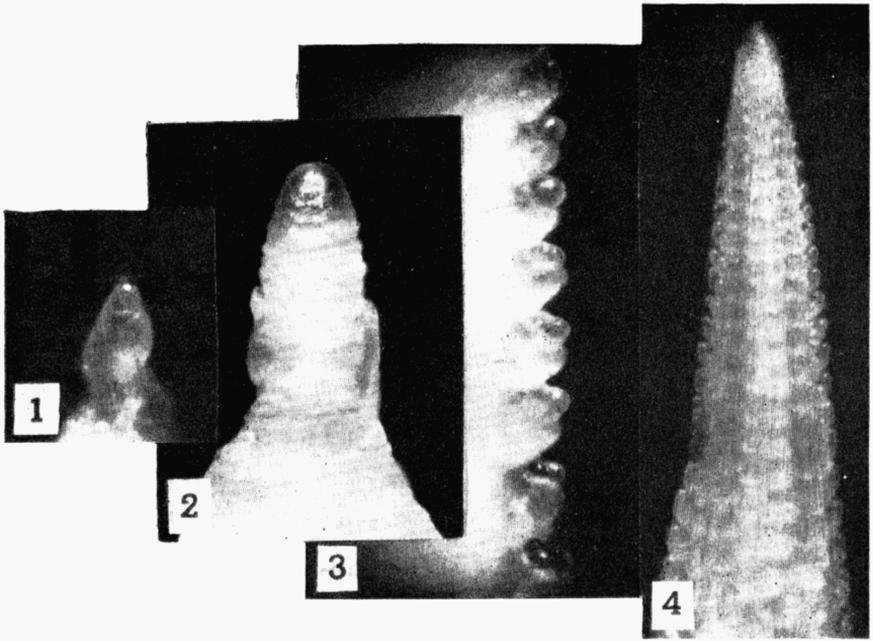
Initial stages of tassel and ear differentiation in *Zea mays* L. from common primordia.

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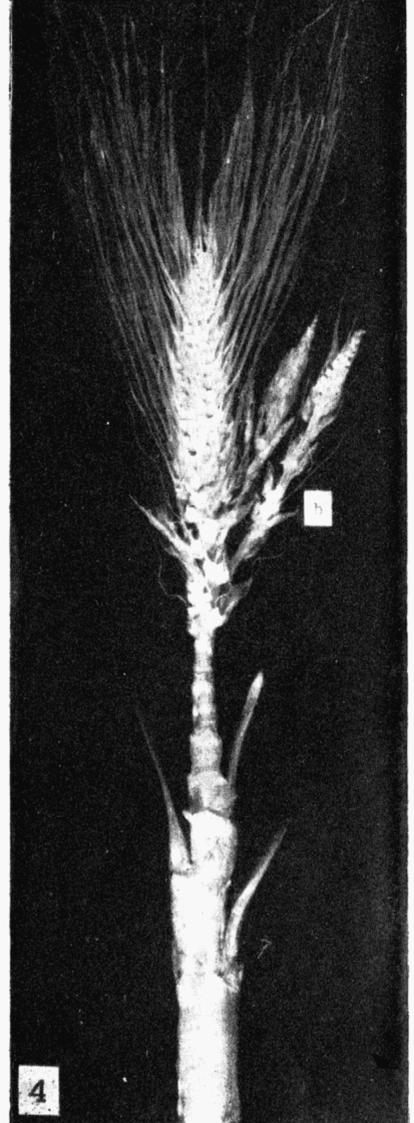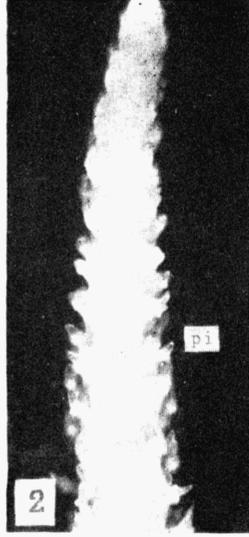
Transformation of staminate tassel primordia into pistillate ear after the application of maleic hydrazide.

Z. Sladký: Experimental Study of Floral Morphogenesis II. Contribution to the Morphology of Infiorescence in *Zea mays* L.
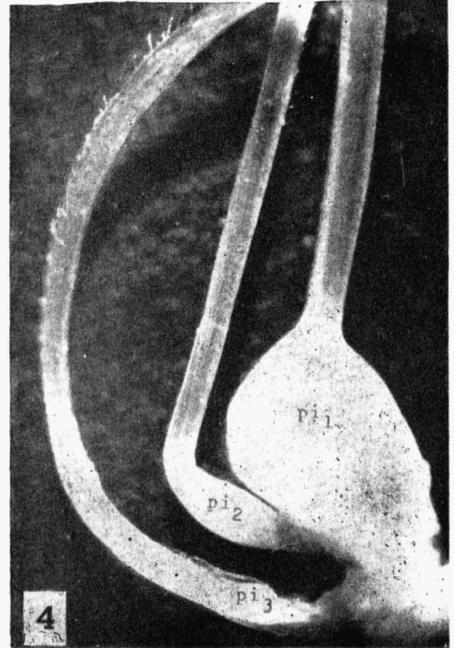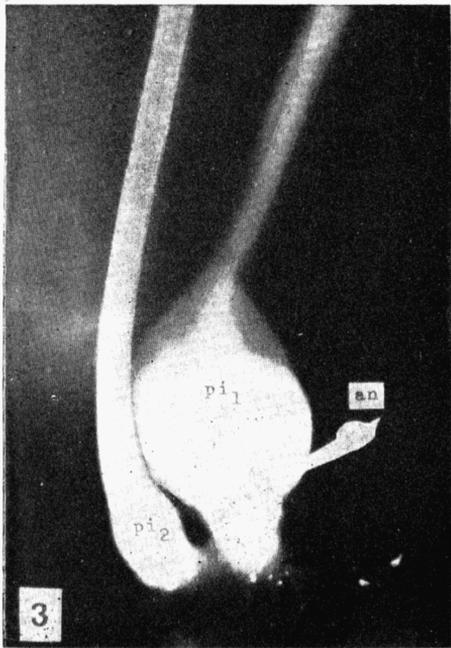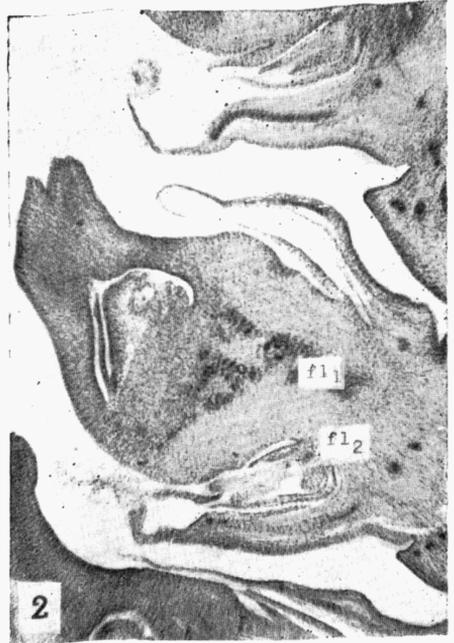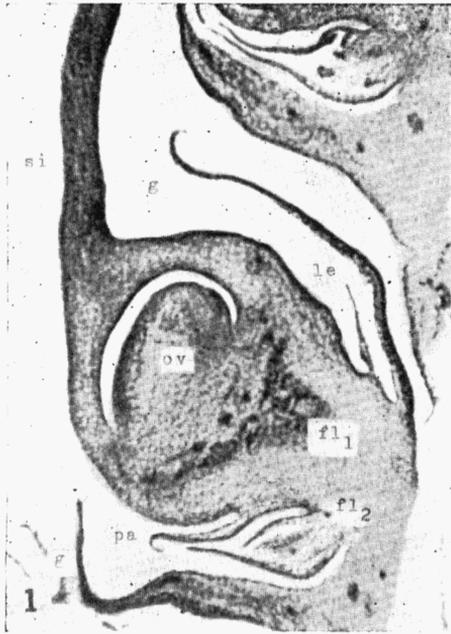
Anatomical and morphological structure of the spikelets whose differentiation process was disturbed in the more advanced stages.

Z. S l a d k ý: Experimental Study of Floral Morphogenesis II. Contribution to the Morphology of Inflorescence in *Zea mays* L.
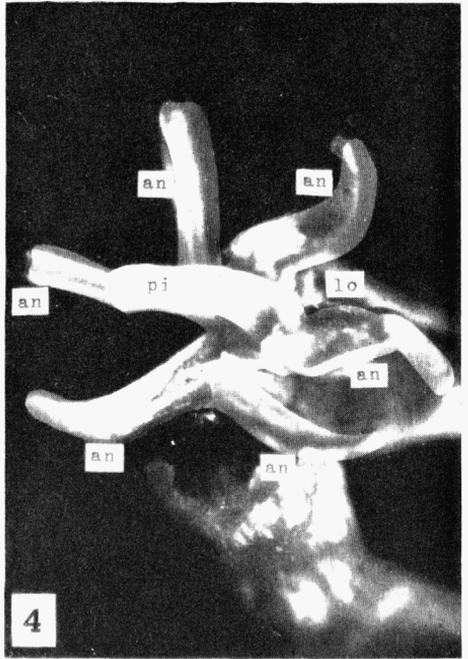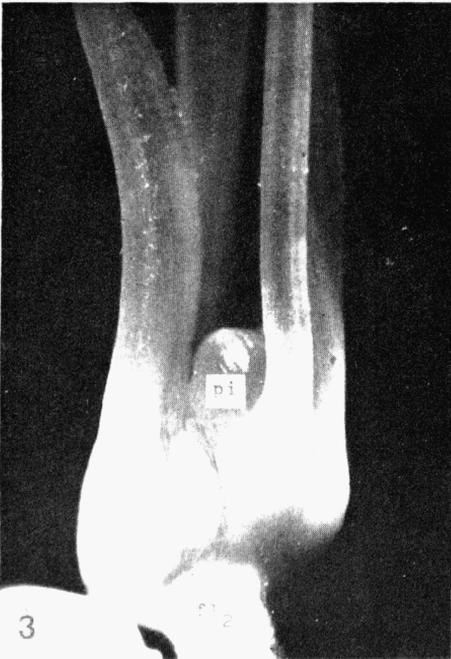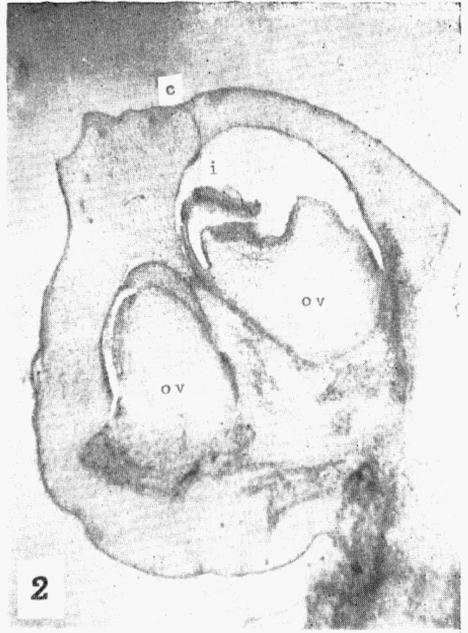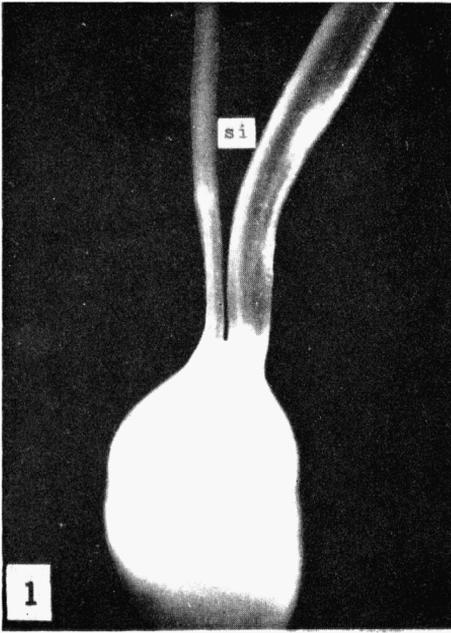
Further types of abnormalities which originated after interventions in various stages of floral and spikelet primordia differentiation.

an = anther; b = long branch; c = carpel; fl_1 = upper flower; fl_2 = lower flower; g = glume; i = integument; le = lemma; lo = lodicule; pa = palea; pi = pistil; ov = ovule; si = silk, stigma.

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