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Role of Growth Regulators in Differentiation Processes of Maize (*Zea Mays* L.) Organs

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Abstract. The following paper deals with the character of endogenous auxins and gibberellin-like substances in the maize tassel and ear primordia during differentiation. Using bioassay the character of substances extracted from tassel primordia, internodes below the tassel, ear primordia and stem base was determined and correlated with the course of morphogenesis and differentiation. A low level of auxins and a high content of gibberellin-like substances accompanies the differentiation of terminal tassel. The differentiation of an ear is associated with an increment in auxin content while the level of gibberellin-like substances decreases. The character of growth substances in primordia remains practically unchanged in the course of further differentiation. The inhibitions appear in the plant and probably start numerous morphological reductions in the pistillate inflorescence structure or inhibit the growth of lateral primordia on the stem *etc.* The treatment of plants with maleic hydrazide at the beginning of tassel differentiation shifts the normal levels of endogenous regulators and brings about the transformation of tassel primordia into an ear. This transformation is accompanied by a marked rise in gibberellin-like substances, by an increment in auxins and the appearance of inhibitors.

The coordination of all the processes and the harmonic step by step development in the morphogenesis of plant organs is quite a puzzling phenomenon. We do not understand the mechanisms governing cell division and ontogenetical development at the moment. The problem lies evidently in the difficulty of characterizing the levels of organization where the processes are taking place (ZUBAY 1964). Morphogenesis may be considered as a result of many biochemical processes anchored in the genetical backround of the plant. HESLOP-HARRISON (1964) points out that the level of growth regulators in the meristem before differentiation influences the pattern of further development. In our previous work (SLADKÝ 1966) we paid attention to the role played by substances of auxin character in the differentiation of tassel and ear primordia. The aim of our present work was to study the link between endogenous auxins and gibberellins and some morphogenetical and differentiation processes in maize. We were also interested in the role played by these substances in the induction of abnormalities.

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Material and Methods

The single hybrid of maize (WH × W9) was used in the experiments. In the years 1965 to 1967 plants were grown in the field from the beginning of May to the end of September. The auxins and gibberellin-like substances were determined in the tassel primordia(1), in the internodes below the tassel (2), in ear primordia (3) and in the internodes at the stem base (4). The first series of experiments was carried out with plants 35 ± 3 days old with terminal tassel primordia 1·5 cm. long and the phase of the beginning of staminate floret differentiation. Plants 49 ± 3 days old were used for the second series of experiments. In these plants the ear primordia in the middle of the stem were 1·5 cm. long and they reached the phase of the beginning of pistillate floret differentiation. The changes in growth substance content after 0·1 per cent maleic hydrazide treatment were determined in plants 49 ± 3 days old. When repeating the experiments special attention was paid to use the primordia not only of the same age but also of the same developmental phase.

Peroxide-free ether was used for extraction according to BENTLEY and HOUSLEY (1954) and KEFFORD (1955). The original method was modified in some details. 10 g fresh weight of excised primordia were homogenized and extracted three times with 100 ml cooled ether. The combined extracts were concentrated and the residue dissolved in 1·5 ml ethanol. 0·5 ml was applied to Whatmann No. 1 paper and a mixture of isopropanol-ammonia-water (8 : 1 : 1) used for chromatography. 1·0 and 0·1 ppm solutions of IAA were used as a standard. Chromatograms were divided into ten zones and eluted using 3 per cent sucrose. Oat *(Avena sativa* L.) cv. Český žlutý was employed for coleoptile segment bioassay. The segment's elongation was expressed in histograms as percentage of control elongation. Each histogram represents the mean value of at least three experiments with 3·3 g primordia fresh weight.

Gibberellin-like substances were determined in 2 g fresh weight of appropriate organs using TLC technique according to SEMBDNER, GROSS and SCHREIBER (1962). The volume of extracts was reduced by evaporation of methanol the residue dissolved in 0·5 ml ethylacetate and 0·3 ml applied on silicagel G (Merck) thin layer. Gibberellic acid of Polish origin (Gibreskol) in concentration 0·1 and 1·0 ppm was used as a standard. Chromatograms were developed in solvent system chloroform-ethylacetate-ice acetic acid (60 : 40 : 5) and the gibberellin-like activity of different zones examined by lettuce hypocotyl bioassay (cv. Stupický Kamenáč). The results of the analysis are expressed in histograms as a percentage of control elongation. Each histogram represents the mean value of at least three experiments with 2 g fresh weight.

Plants were sprayed with 0·1 per cent maleic hydrazide prior to differentiation of staminate spikelets in the tassel primordia. The treatment was repeated after six days. The course of morphogenesis and differentiation was followed simultaneously with the changes in growth regulators level. The photograph represents differentiation of an ear and the numbers indicate regions of sampling for endogenous regulator extraction.

Results

The character of auxins and gibberellin-like substances at the beginning of spikelet and floret differentiation in a tassel is represented in Fig. 1. The upper histograms present data on auxin bioassay, the lower ones on gibberellin bioassay.

The beginning of terminal tassel differentiation is associated with low auxin level (1). In the internodes below the tassel (2) there are regions of stimulative

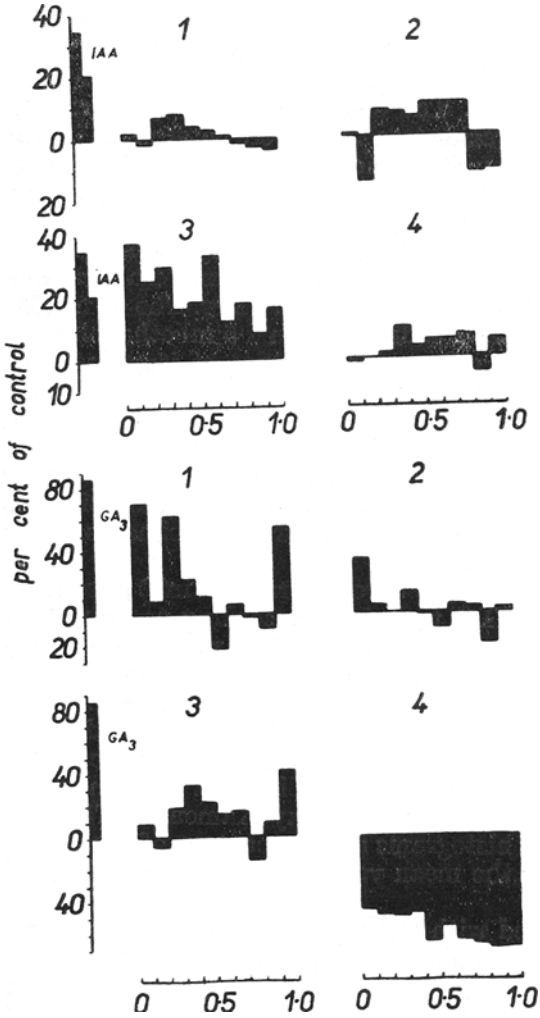

Fig. 1. Character of endogenous regulators in maize primordia prior to differentiation of staminate florets of the tassel. The histograms on the left side above represent auxins, the histograms below gibberellin-like substances in tassel primordia (1), in internodes below tassel (2), in ear primordia in the middle part of the stem (3) and at the stem base (4). The effect of 1·0 and 0·1 ppm of appropriate standard is indicated on the ordinate.

as well as inhibitive character especially at Rf 0·2, 0·9 and 1·0. Ear primordia (3) contain substances of stimulative nature. Rf 0·5 and 0·6 correspond to IAA in a concentration higher than 1 mg/l. There is only low auxin activity at the stem base (4).

The lower histograms represent results on gibberellin-like substance estimation. As shown in histogram 1, the highest level of gibberellin was found in the tassel primordia. The activity at Rf 0·3 and 0·4 corresponds to the effect of approximately 0·1 mg/l GA_3. There are zones of stimulative character even at the start and in the front of a chromatogram. The internodes below the tassel (2) contain no substances causing lettuce hypocotyl elongation. The ear primordia (3) contain less substances of stimulative character than tassel primordia. Substances from stem base (4) are exclusively of inhibitive character.

Fig. 2 shows the result of analyses done 14 days later, at the time of spikelet and floret differentiation in the ears. There exists a certain correspondence with the previous results, the inhibitions being more pronounced.

The level of auxins in the tassel primordia (1) remains practically unchanged. In the internodes below the tassel (2) quite a marked inhibition appears at Rf 0·2, 0·4 and 0·5. The auxins in ear primordia (3) even increased. The auxin effect is quite clear-cut at Rf 0·5 and 0·6 where the stimulation corresponds to an IAA concentration of 6·5 mg IAA/10 g fresh weight of ear primordia. Slight inhibition at Rf 0·2 appeared. The situation at the stem base (4) remains practically unchanged.

The lower histograms again represent gibberellin-like substances. In the tassel (1) the stimulation at Rf 0·1 and 0·4 is accompanied by pronounced inhibition at Rf 0·6. There is a rise in inhibitions even in the internodes below the tassel (2), at Rf 0·1 and especially at 0·5 to 0·7. In ear primordia (3) the gibberellin-like substances tend to decrease and zones with inhibitive character appear. At the stem base (4) the inhibitions are less marked in comparison with the previous situation, neverthless one half of Rf zones reveal an inhibitive character.

The changes in endogenous regulator level caused by two maleic hydrazide treatments prior to terminal tassel differentiation are shown in Fig. 3. It follows from histograms, that the treatment brought about many fundamental changes. Inhibitions appear in the tassel primordia and a slight increment in auxins may be observed. There is a drop in inhibitions in the internodes below the tassel (2). The content of substances with stimulative character in ear primordia (3) decreases and new inhibitive regions appear. At the stem base (4) zones of stimulative and inhibitive character interchange along the whole chromatogram. The comparison with Fig. 2 shows clearly the destructive effect of maleic hydrazide on the normal level of endogenous substances with auxin character.

In the lower histogram the effect of maleic hydrazide on the level of gibberellin-like substances is shown. The effect is even more pronounced than in the previous case and a general increment in substances of stimulative character may be observed. The highest values were found for tassel primordia where zone 1 surpasses the activity of 1 mg/l GA_3. Rf 0·3 and 0·4 correspond approximately to the effect of 0·1 mg/l GA_3. There is an increased stimulative activity even in the internodes below the tassel (2) and in ear primordia (3)

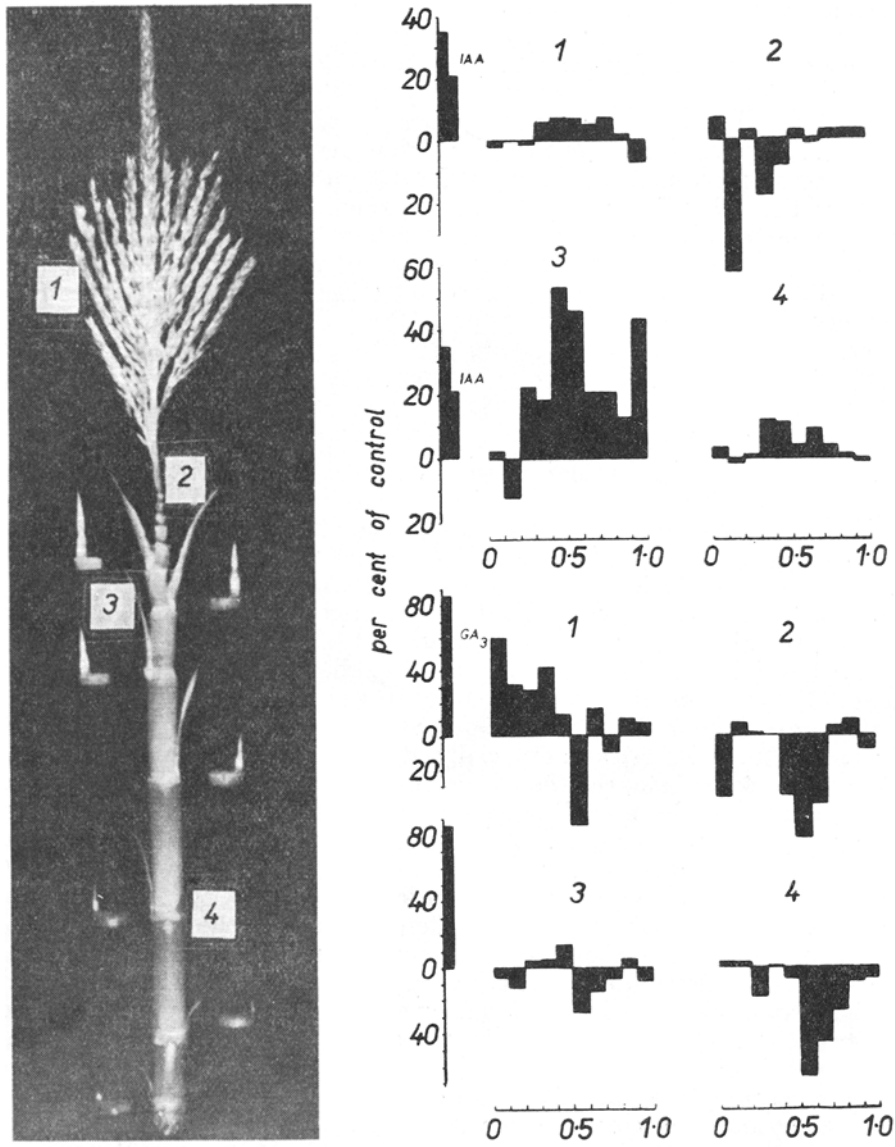

Fig. 2. Character of endogenous regulators in maize primordia 14 days after first determination *i.e.* at the time of ear pistillate florets differentiation. The data are presented in the same way as in Fig. 1. The phase of development is characterized by a photograph of a maize plant with differentiated tassel and pistillate florets of the ear in the course of differentiation.

where already some inhibitions may be found. There was no inhibition in the samples from the stem base as in the previous cases by control plants.

From the morphological point of view maleic hydrazide brought about the

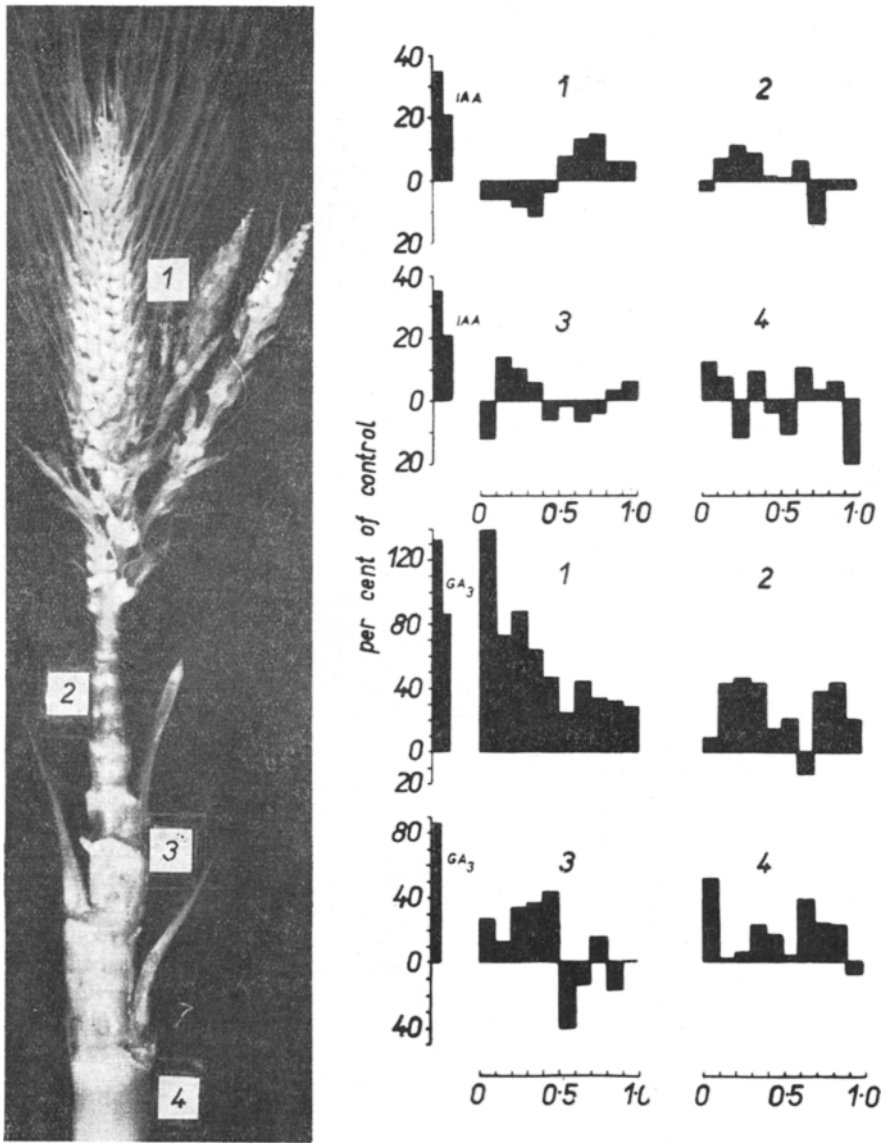

Fig. 3. Treatment with 0·1 per cent maleic hydrazide causes changes in the level of endogenous auxins (above) and gibberellin-like substances (below) in differentiating tassel primordia (1), in internodes below the tassel (2), in ears in the middle part of the stem (3) and at the base of the stem (4). The photograph shows transformation of the tassel in pistillate ear.

feminization of tassel florets and even the tassel primordia were transformed into a typical ear (Fig. 2). The appropriate treatment with maleic hydrazide elongates internodes below the tassel and causes uniform growth of several ears on the stem.

Discussion

The results indicate that a high degree of specialization in the structure of maize organs is based on a perfectly developed system, with auxins, gibberellin-like substances and inhibitors playing an important role. The method of bioassay used for evaluation of chromatographic zones enables us to characterize endogenous substances and yields data for elucidation of the pattern of different primordia differentiation. No definite conclusions may be drawn in respect to possible interaction between substances with stimulative and inhibitive character and of free and bound forms of growth substances as conceived by LIBBERT (1954).

The results indicate that the level of growth regulators is rather constant during the whole course of differentiation. We found that a differentiation of pistillate inflorescence is accompanied by a higher auxin level which is in accordance with our previous data (SLADKÝ 1966) and those of CONRAD and MOTHES (1961), who have found 30 times more auxins in pistillate than in staminate hemp plants.

There is a rise in inhibitions during maize primordia differentiation. Nothing definitive can be said about the character of these inhibitions. The difficulty lies, in the first place, in a lack of adequate methods and no standards for comparison are available. It seems that the inhibitors play a role at least of similar importance to that of stimulators. According to HESLOP-HARRISON (1964) the feminization of maize tassel florets by short day is accompanied rather by inhibitor accumulations, than by increment of native auxins level. GALUN (1959), on the other hand, ascribes a limiting role to auxins in the differentiation of cucumber inflorescence primordia. He came to the conclusion that an addition of IAA to the cultivating medium aids the carpel development and inhibits the growth of stamens. In intact cucumber plants gibberellin acts in a way opposite to auxins and inhibits the development of pistillate flowers by the simultaneous enhancement of staminate primordia growth.

PILET's (1961) view on the synergic effect of gibberellic acid and auxin, which is often cited in the literature, is probably more valid for growth than for differentiation. The relation between auxin and gibberellin seems to be rather antagonistic in our experiments. Low auxin level and high gibberellin content is associated with tassel differentiation and the reverse is true for ear differentiation.

The appropriate treatment with maleic hydrazide brings about alteration in the level of endogenous regulators and changes the morphological structure. It seem that the shifts in growth regulator levels are related to morphological changes in a way similar to that in previous cases. This kind of data may be considered as a further indication of a link between endogenous regulators and differentiation processes. In accordance with TELTSCHEROVÁ's findings (1968) we may say that the determination of growth substance level relations is a more important indicator of differentiation than the determination of individual substance concentration.

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Z. SLADKÝ, Laboratoř fyziologie a anatomie rostlin Přírodovědecké fakulty, Brno: **Úloha růstových regulátorů v procesech diferenciace orgánů kukuřice *Zea mays* L.** — Biol. Plant. **11** : 208 až 215, 1969.

Předložená práce sleduje charakter endogenních látek auxinové a giberelové povahy v základech orgánů kukuřice během diferenciace prašníkové laty a pestíkové palice. Biologickým testováním byl zjišťován charakter extrahovaných látek ze základů laty, internodií pod latou, základů palic a baze stébla a je uváděn do vztahu s průběhem morfogeneze a diferenciace. Nízká hladina auxinů a vysoký obsah látek giberelové povahy provází diferenciaci terminální prašníkové láty. Diferenciace pestíkové palice je charakterisována zvýšením obsahu látek auxinové povahy a snížením hladiny giberelinů. V průběhu další diferenciace se charakter látek v základech podstatně nemění. Dochází k narůstání inhibic v rostlině, které pravděpodobně zavádí řadu morfologických redukcí ve stavbě pestíkového květenství, nebo inhibují růst laterálních základů na stéble apod. Postřik rostlin maleinhydrazidem na počátku květní diferenciace laty poruší přirozenou hladinu regulátorů v rostlině a vyvolá přeměnu základů prašníkové laty v pestíkovou palici. Metamorfoza je provázena podstatným zvýšením látek giberelové povahy, stoupnutím hladiny auxinů a výskytem látek inhibiční povahy.

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В работе исследовались эндогенные вещества ауксинового и гиббереллинового характера в зачатках органов кукурузы во время дифференциации метелки и початка. Биотестами установлен характер веществ экстрагированных из зачатков метелки, междоузлий под метелкой, оснований початков и нижней части стебля. Уровень ростовых веществ соотносится с морфогенезом и дифференциацией. Низкий уровень ауксинов и высокое содержание веществ гиббереллинового характера сопровождает дифференциацию верхушечной метелки. Дифференциация початка характеризована повышением веществ ауксинового характера и понижением уровня гиббереллинов. Во время последующей дифференциации характер веществ в основном существенно не изменяется. В растении усиляются ингибиции, которые вероятно вводят ряд морфологических редукций в строении початка или подавляют рост боковых зачатков на стебле и т. п. Опрыскивание растений малеингидразидом в начале дифференциации цветков метелки нарушает естественный уровень регуляторов в растении и вызывает превращение зачатков метелки в початок. Метаморфоз сопровождается существенным повышением уровня веществ гиббереллинового характера, повышением уровня ауксинов и появлением ингибиторов.