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Changes in Endogenous Gibberellins in Plant Organs Producing and Utilizing Photosynthates

J. REPKA and ZUZANA JUREKOVÁ

Department of Plant Biology, University of Agriculture, Nitra*

Abstract. The distribution of endogenous gibberellin-like substances in individual organs of *Zea mays* L., cv. CE 250, plants was investigated during the transition from the vegetative to the generative phase of development. The gradient of the content of gibberellin-like substances and photosynthetic activity in leaf segments was followed in different parts of the leaves, as well as the changes in the content of gibberellin-like substances in leaf segments during an exposure in the light and in the dark. The gradient of the content of endogenous gibberellin-like substances in the leaves, in the stem and in the spike is interpreted in terms of possible relationship of these compounds to the regulation of sink — source.

Growth regulators are thought to be specific compounds regulating plant growth processes. They are generally assumed to be synthesized in one organ and transported to other sites in the plant, where they can activate the processes of organogenesis and growth (KEFELI 1974). Similarly, the generation, distribution and accumulation of photosynthates occurs in plants in the course of plant growth, this being supposed to be one of the possible ways of regulation and control of photosynthetic activity in plants (MOKRONOSOV 1973). For this reason, the role of plant growth regulators has been recently considered in connection with the regulation of the transport of solutes in the plant. MOTHES (1964) has reported that auxins, gibberellins, cytokinins and naturally occurring inhibitors exert regulatory effects on the flow of solutes and water. They either enhance or slow down the flow of nutrients to that plant part in which they accumulate (fruits, seeds, tubers).
The hypothesis on hormonal activation of the "sink" is further supported by the results of NÖSBERGER and HUMPHRIES (1965), SETH and WAREING (1967) and WAREING and PATRICK (1975).

This effect has also been demonstrated with exogenously applied growth regulators (LEPP and PEEL 1970, POSKUTA *et al.* 1975). These and other papers have confirmed the influence of growth regulators on plant photosynthetic activity and have drawn attention to the possibility of the translocation of solutes between the source and the site of utilisation being controlled by growth regulators (MARCELLE and OBEN 1973, OBEN and MARCELLE 1975, WARDLAW and MONCUR 1976).

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* *Address:* 949 01 Nitra, Czechoslovakia.

Continuing in the investigation on the heterogeneity in the content of gibberellin-like substances in different plant organs found by us in winter wheat (JUREKOVÁ and REPKA 1973), we present in this paper the results dealing with the changes in the content of these substances in different organs of maize plants with regard to the possibility of these changes being connected with the process of formation and utilisation of photosynthates in growing plant organs.

Material and Methods

Maize (*Zea mays* L. cv. CE 250) plants served as the experimental object. Plants were grown in vegetation vessels under natural conditions with sufficient mineral nutrition and water supply.

Analyses

The analyses were carried out in the course of the vegetation period with special regard to the transition from the vegetative to the generative phase of development. The content of endogenous gibberellin-like substances was followed in individual plant organs (*i.e.* in the leaves, internodes, spike and panicle). The photosynthetic rate was determined in different leaf parts according to the increment in dry matter of leaf segments in a rotation exposure device, after 5 h exposure at 22 °C and irradiance of 56 W m^{-2}, the time of exposure in darkness being also 5 h at 22 °C.

Extraction and Determination of Endogenous Gibberellin-like Substances

Free gibberellin-like substances were extracted from acid ethylacetate fraction. Combined extracts were evaporated and separated chromatographically on a thin layer of Silicagel. Bound gibberellin-like substances were determined in hydrolysed aqueous phase of the remainder left after the extraction of the free forms. Acetate extracts were combined, evaporated and separated (JUREKOVÁ and REPKA 1973).

The detection of the substances was carried out by means of the bioassay according to FRANKLAND and WAREING (1960) as modified by KREKULE and TELTSCHEROVÁ (1963).

Results

Considerable qualitative and quantitative differences can be found in the content of endogenous gibberellin-like substances between the individual plant organs at each stage of plant development as well as changes in the content and transformation of these compounds. That is confirmed by the data on the content of endogenous gibberellin-like substances in vegetative (leaves and stem internodes) and generative (panicle, spike) organs at the stage of transition from the vegetative to the generative phase.

The analysis of the content of endogenous gibberellin-like substances in the leaves (Fig. 1) shows that two gradients of gibberellin content are formed at the stage of nine leaves (at a time when no generative organs were yet formed on the plants). Lower leaves (3, 4, 5) and the upper leaf contain a low level. The leaves situated in the middle (6, 7, 8) show a gibberellin level raised by 100 to 150 %.

From the qualitative point of view, stimulatory substances occurred only in the Rf region 0.1, the other regions, especially Rf 0.3 and 0.6–0.9, being occupied by inhibitory substances. The trend in the upper leaf was the

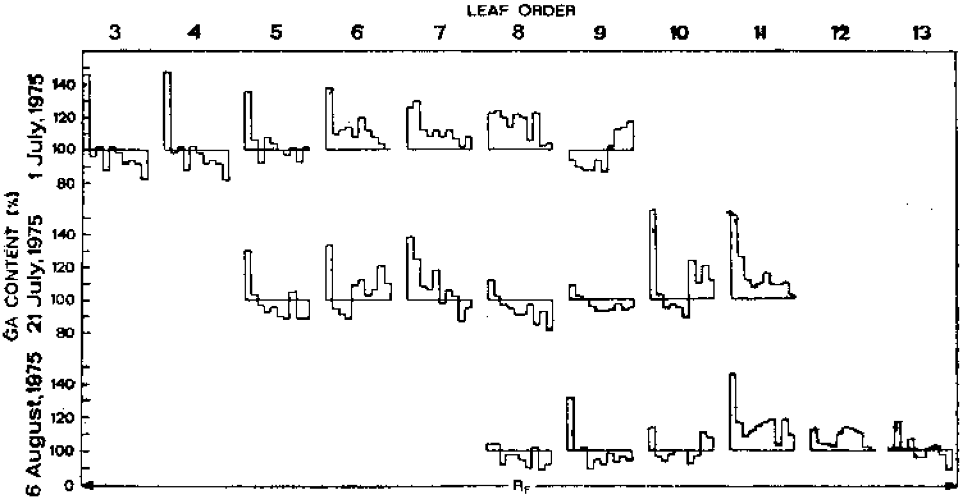

Fig. 1. Changes in the content of free endogenous gibberellin-like substances in the leaves at the stage of the formation of generative organs. Abscissa: Rf values 0.1—1.0. Ordinate: GA content [%].

opposite: compounds of stimulatory nature were in the Rf region 0.7—1.0, of inhibitory nature in the region 0.1—0.6. The leaves rich in gibberellin-like substances (6, 7, 8) did not contain any inhibitors.

The gradient of the content of gibberellin-like substances in the leaves changes in the subsequent period (21 July) though the differences in the content can again be observed. A loss of gibberellin-like substances occurs in the 8th and in the 9th leaf, with other leaves (10, 11) containing a large amount of the investigated substances.

These trends cannot be explained by changes in growth, because actively growing leaves were always analysed in each investigated period as can be supported by the following comparison (Table 1): The fifth leaf increased its weight three times, the sixth 2.8 times, the seventh 2.9 times and the eighth 3.5 times. It can be stated that with approximately equal accumulation of dry matter, the level of gibberellin-like substances was decreased most in the eighth leaf.

TABLE 1

Increment in dry matter in individual leaves in the course of the investigated period

Analysis	Dry matter in g per plant of the leaf Nr.						
	5	6	7	8	9	10	11
1 July	0.410	0.590	0.609	0.480	0.230	0.080	0.035
21 July	1.268	1.700	1.813	1.703	1.500	1.273	0.912
ΔW [g]	0.858	1.110	1.204	1.223	1.270	1.193	0.877
W [%]	309.2	288.1	297.7	354.7	652.0	1591.0	2605.0

The highest increase in weight was found in the tenth and the eleventh leaf (16 times and 26 times respectively) and these leaves also contained a large amount of gibberellin-like substances.

The leaves 8 to 13 were analysed at the stage of spike formation (8 August). Among these the eighth and the tenth leaf contain a low level and similarly also the thirteenth leaf. At the same time a high level of gibberellin-like substances occurs in the eleventh and in the twelfth leaf which is not accompanied by the presence of substances of inhibitory nature.

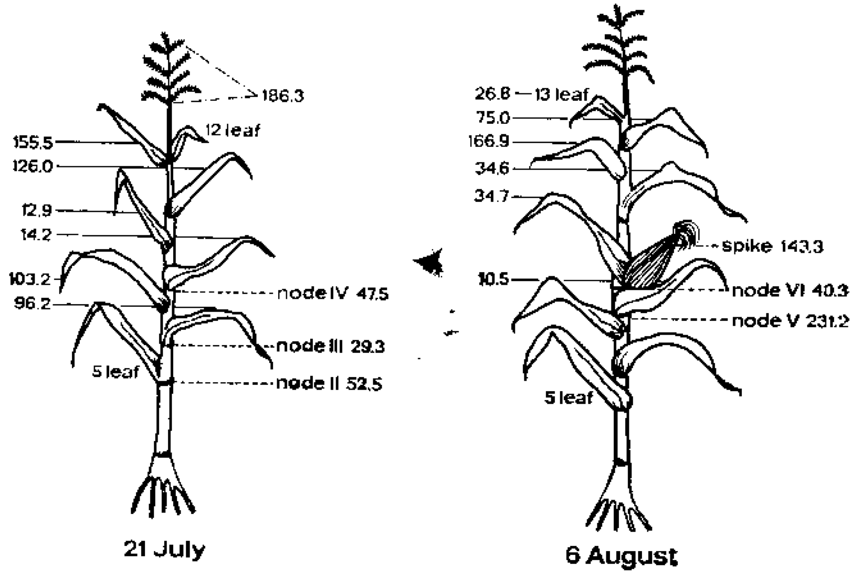

Fig. 2. Diagrammatic representation of the values of total content [%] of gibberellin-like substances in some maize organs before (July 21) and after (August 6) the formation of the spike.

Such a predivision and level changes do not confirm unambiguously that the dynamics in the content of the investigated substances is connected with growth processes of the given organ. For example the sixth and the seventh leaf had elevated levels of gibberellin-like substances in comparison to the eighth and the ninth leaf which were growing, but the gibberellin level of which decreased and in which inhibitory substances also appeared. By contrast, vigorously growing leaves (10, 11) contained a large amount of gibberellin-like substances. The cause of the loss of gibberellin-like substances in the eighth and the ninth leaves is unknown. A certain explanation is offered by the data on changes in the content of gibberellin-like substances in developing generative organs.

The spike was formed over the eighth leaf in the period between 1 July and 6 August. It can therefore be assumed that the changes in the content of the growth regulator in the 8th leaf are connected with this process. If we compare the sum of the activities of all the positive Rf, we obtain the following pattern of the content of endogenous gibberellin-like substances. The eighth leaf contained a high level (149 %) on 1 July. The panicle was formed on 27 July and the content in the eighth leaf dropped to 14 %. The content in the panicle was 186%. After the formation of the spike (6 August), the distribution of gibberellin-like substances was as follows: the eighth leaf 10%, the internode to which the spike is attached 231% and the spike 143%. These changes are represented schematically in Fig. 2. This example and further analyses have confirmed that gibberellin-like substances disappear from the leaf behind which the spike is formed, with their content increasing in the internode and in the spike. Other leaves situated in the proximity of the spike (9, 10) also have a low content of the investigated substances, whereas the leaves situated higher on the stem (11, 12) contain large amounts of these substances. This differentiation is not directly connected with decreased physiological performance of the investigated leaves. This also follows from the comparison of photosynthetic activity in segments of leaf tissue with their content of gibberellin-like substances (Table 2).

TABLE 2

Changes in the content of endogenous gibberellin-like substances (GA) and photosynthetic activity of the leaves (PA) at the stage of spike formation

Date of analysis	Leaf	GA [%]	PA [mg dm^{-2} h^{-1}]
1 July	6	125.6	7.5
	7	125.6	6.7
	8	149.0	6.1
21 July	8	14.2	9.0
	9	12.9	8.5
	10	126.0	8.0
6 August	8	10.5	8.9
	9	34.7	8.7
	10	34.6	8.3
	11	166.9	9.1
			PA s.d. 0.05—0.73
			0.01—1.21

The data indicate that even when the leaf loses its gibberellin-like substances, its activity does not diminish. The decrease in the content of these substances in assimilating tissue cannot be connected with the inhibition of the function "source". To verify if the content of endogenous gibberellin-like substances is connected with the photosynthetic activity in assimilating tissue, we analysed a simpler model — the leaf. We started from the knowledge on the photosynthetic heterogeneity of the leaf in its basal and apical parts. When the leaf was divided into four equal parts and the content of gibberellin-like substances and photosynthetic rate were determined in them, an interesting pattern of the investigated variables was obtained (Fig. 3a). The results have shown that the gradient of the content of gibberellin-like substances has a decreasing trend from the apical to the basal part and does not fully conform with the FAS values. The data presented here indicate that considerable differences in the distribution or synthesis of growth regulators occur even in such an experimental object as a single plant organ (*i.e.* the leaf). The interpretation of such a distribution is difficult. The basal part of the leaf is either the site of active leaf growth, or the site through which assimilates and growth regulators are transported to the internode. In the first case, it should be the site of consumption (sink) which should be manifested by a high content of gibberellin-like substances. To confirm this assumption, we analysed a young growing leaf the basal part of which was already fully developed. The distribution of gibberellin-like substances (Fig. 3b) again confirmed that the lowest content of the substances is in the

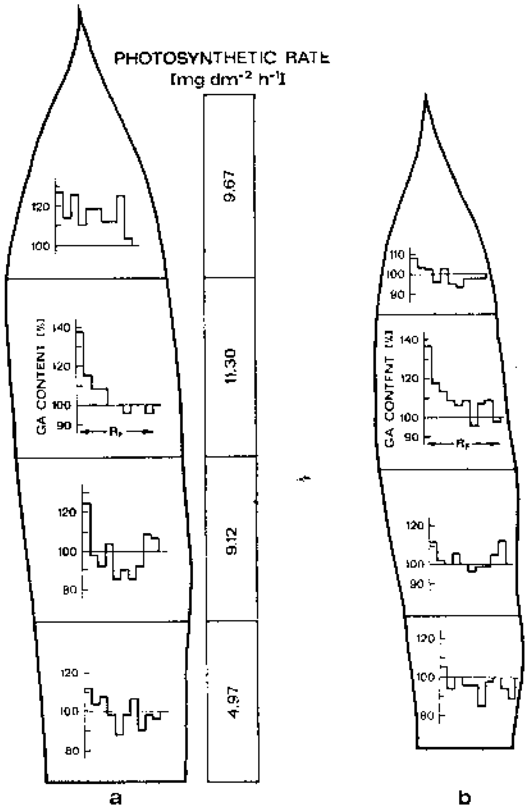

Fig. 3. The gradient of the content of free endogenous gibberellin-like substances and of photosynthetic rate in different parts of a mature leaf (a) and the gradient of the content of endogenous gibberellin-like substances in different parts of a growing leaf (b).

basal part of the leaf, the highest content being in the part with supposedly the highest photosynthetic activity. It is an example of a high content of gibberellin-like substances in the source and of a low content of gibberellin-like substances in the sink.

To elucidate better the possibilities of a direct relationship between the content of gibberellin-like substances in the leaf tissue and its photosynthetic activity, we measured the content of the substances in leaf segments exposed to light (photosynthesizing) and in leaf segments kept in darkness (without photosynthesis). The results of one example of the measurement are presented in the histogram (Fig. 4). The experiment shows that the content of gibberellin-like substances decreased after a 5 h exposure of leaf tissue to the light, mainly in the Rf region 0.4—1.0. The measurement was repeated several times with the resulting decreased content of the investigated substances in photosynthesizing segments to 43%, 33% and 14% in relative values. At the same time an increase always occurred in gibberellin content in the segments kept for 5 h in the dark.

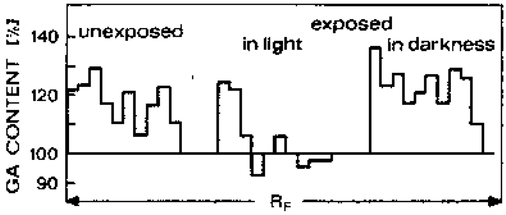

Fig. 4. Changes in the content of endogenous gibberellin-like substances in leaf segments exposed to light or kept in darkness. Abscissa: Rf values 0.1—1.0; Ordinate: GA content [%].

The loss of gibberellin content in photosynthesizing tissue is more connected with the chloroplast fraction of the leaf, which follows from the analysis of gibberellin content of leaf homogenates obtained during chloroplast isolation (Table 3). The chloroplasts were isolated from leaf segments before and after a 5 h exposure to light.

The data given in the table show that if photosynthesis takes place together with restricted transport of the photosynthates, considerable qualitative structural changes in the fractions with gibberellin-like activity occur. The level of free substances decreases and the level of bound forms increases. The chloroplasts are characteristic with the lowest loss of the free and with the largest enrichment with bound forms of gibberellin-like substances.

Table 3

The content of free and bound endogenous gibberellin-like substances (GA) in leaf homogenates and in the chloroplasts

	Content of free GA		Content of bound GA	
	before exposure	after	before exposure	after
Rough cellulose fragments	84.90	11.00	11.40	50.80
Sediment after 3 min centrifugation	20.10	6.60	—	11.80
chloroplast fraction	53.90	50.80	75.40	183.70

Discussion

The results presented in this paper raise some questions about the functional role of gibberellins in the regulation of plant growth processes and about the relationship between their content and the processes taking place in the very source of photosynthates.

If any place at which assimilates are deposited is considered to be a sink (BEEVERS 1969), then a growing leaf produces photosynthates for itself and also for other plant parts. Photosynthates are first used at the site of production and no special mechanism determining the direction of transport to the site of utilisation is necessary. From this point of view it is, however, very difficult to explain the large gradient in the content of gibberellin-like substances in different parts of the leaf. The other aspect of hormonal rearrangement of the leaf can be connected with the moment when the leaf starts to produce photosynthates predominantly for other plant parts. The loss of gibberellin-like substances may be a signal for this moment. On the other hand, the accumulation of growth regulators could form a concentration gradient which could direct the flow of photosynthates to the given place. The mechanism of this connection is not clear. A growth regulator can activate a growth centre, stimulate attractive activity for photosynthates in competition with other plant parts, increase the mobilisation ability of the sink, *etc.* (WAREING and PATRICK 1975). In this case it is possible to speak of the movement of photosynthates directly into plant parts with high concentration of growth regulators and thus of growth regulator controlled transport. The distribution of gibberellin-like substances in the leaf, internode and in the spike indicates such a function also for gibberellin-like substances.

The high content of gibberellin-like substances in the upper leaves can prove from this point of view that the leaves themselves are still a sink. It is not clear, however, if a high content of these substances in the leaf only secures a high growth activity of the leaf or if the leaf is also a producer for other organs. However, is it possible to consider the leaf to be a source of gibberellin synthesis without any regard to its function as photosynthate producer? The transformation of the free forms into the bound ones, when leaf tissues are exposed and transport does not take place, testifies against this assumption. On an intact plant, photosynthesizing leaves with high and low gibberellin content can be found (leaf 8 and 10 on 21 July, or leaf 8 and 11 on 6 August). That means that the given leaf is an active producer of both the growth regulator and photosynthetic products. It can be assumed that the given leaf saturates the requirements of gibberellins in the internode and in the spike for the maintenance of a concentration level necessary for the activity of the growth centre.

The content of gibberellin-like substances in the chloroplast fraction is a special problem. STODDART (1968) draws attention to it and the possibility of gibberellin synthesis taking place in the chloroplasts (STODDART 1969). RAILTON and WAREING (1973) confirmed that short-day treatment resulted in a decrease in gibberellin content in the chloroplasts. Further elucidation of the possibility of the synthesis of these substances occurring in the chloroplasts and the synthesis being connected with photosynthetic activity of the chloroplast would bring nearer an understanding of the functional bonding of gibberellins in the regulatory mechanism determining the source — sink relationship.

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