Histology and Gibberellin Levels in Healthy and Diseased Maize (*Zea mays* L.) Roots Affected by "Mal de Río Cuarto"[1]

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ABSTRACT

The histology of roots of healthy maize plants and those affected by "Mal de Río Cuarto," was studied in correlation with their endogenous levels of free gibberellins. The results obtained indicate that the maize rough dwarf virus (MRDV) produces severe alterations in the central cylinder, lignification of that structure and the endodermis, and abnormal lateral roots with presence of abortive ones. Gibberellin (GA) analyses were performed on sequential gradient-eluted reversed-phase C_{18}-HPLC, and isocratic eluted Nucleosil-HPLC, and assayed with the dwarf rice bioassay. Diseased roots showed quite different GA-like substance profiles compared with those determined for healthy roots. These results indicated that MRDV produces histophysiological modifications in maize roots, also affecting GA metabolism.

COMPENDIO

En el presente trabajo se estudió la histología de raíces de plantas de maíz sanas y las atacadas por el Mal de Río Cuarto, en correlación con los niveles endógenos de giberelinas en sus formas libres. Los resultados obtenidos indican que el virus del enanismo áspero en maíz (MRDV), agente de la enfermedad, produce severas alteraciones en el cilindro central, lignificación de dicha estructura y de la endodermis, y raíces laterales anormales con presencia de raíces abortivas. El análisis de giberelinas se llevó a cabo mediante gradientes secuenciales sobre HPLC fase reversa, C_{10} y Nucleosil, y bioensayo de arroz enano cv. Tanginbozu. Las raíces de las plantas afectadas mostraron un perfil muy diferente al obtenido de raíces de plantas sanas. Estos resultados señalan que el virus MRDV produce modificaciones histofisiológicas en las raíces, afectando probablemente el metabolismo de las giberelinas.

INTRODUCTION

A maize (*Zea mays* L.) disease called "Mal de Río Cuarto" was recorded for the first time during 1976 near Río Cuarto, Córdoba, Argentina. The pronounced damage caused by the disease on some plants produced great concern to the growers, especially as seed losses were up to 90% during 1979, and almost 80% in 1981 and subsequent years.

The characteristic symptoms of the disease described by Nome *et al.* (20), Lenardón and March (14) and Lenardón *et al.* (15), are short internodes, small leaves with enations, and general stunting. Stems appear flattened and glassy. The size of early-infected plants is reduced by about one third of normal ones. Proliferation of ears, two or three ears with almost no kernels on the same node, and proliferation of small tassels with incomplete male inflorescence can also be observed; male flowers are sterile, erect and small; and the volume of the radical system is usually reduced.

Nome *et al.* (20) related this disease to the maize rough dwarf virus (MRDV). They found virus-like particles present in the parenchymatic phloem cells of

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[1] Received for publication 9 February 1990.
This work was supported by a grant from CONICET, CONICOR and 477 PROGRAM from Río Cuarto University. The authors wish to thank Ings. G. March and A. Marinelli of the Agronomy Faculty of Río Cuarto University for their kind help in the identification of infected plants in the field; and Profs. R.P. Pharis and E. Yeung of the University of Calgary, Canada, for their advice.

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roots and stems. Milne *et al.* (16) and Milne and Boccardo (17) subsequently confirmed this finding.

As one of the main symptoms of "Mal de Río Cuarto" is the shortening of internodes (dwarfism) and a reduced radical system, MRDV might affect the hormonal regulation of growth, altering synthesis, metabolism and/or transport of hormones. Short internodes, general stunting and small leaves are characteristics of lack of GA's, all symptoms of plants infected by MRDV.

Several GAs have been identified in tassels and shoots of maize: GA_1, GA_8, GA_{12}, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44} and GA_{53} (8, 9, 10). It has recently been suggested that GAs may regulate normal root growth at a much lower concentration range that shoot growth; also, roots synthesize GAs which are transported to shoots (3, 5, 6, 18, 23).

There are only a few studies on growth regulators in infected plants. Russell and Kimmins (24) showed a decrease in the level of a substance GA_3, as in barley yellow dwarf virus-infected plants, suggesting that dwarfism was a result of reduced mitotic activity related to the lowered level of endogenous gibberellins. Application of GA_3 to infected individuals reversed the dwarfing effect and increased cell elongation. However, Bailiss (1) studied the relation between GAs and the "early disease syndrome" of aspermy virus in tomato, reporting that growth decreased by virus infection could not be overcome by exogenous GA_3.

In a previous work (4), MRDV infection was shown to mainly affect vascular tissues of first and second basal stem nodes and internodes. Phloem presented hyperplasia with proliferating cells which had lost their structure and typical disposition. Also a xylem mass in an abnormal position was observed, with abundant vessel members and tracheids of thick walls and often obliterated by an unknown substance. The analysis of free active GAs proved that MRDV would affect the endogenous levels of these phytohormones; however, significant differences were not found in relation to conjugated forms.

The objectives of this study were to compare the morphological and histological alterations caused by MRDV in healthy and affected roots of maize plants; examine the changes of the endogenous free ethyl acetate-soluble GA-like substances in both, infected and healthy tissues, and the correlation between the hormonal level and the dwarf symptom.

MATERIALS AND METHODS

Roots from healthy and MRDV-infected plants of maize (*Zea mays* L.) (hybrid Morgan 400), were collected from the field in Río Cuarto. Two samples were taken, the first in December 1986, when enations in leaves were evident and plants were approximately 1.20 m high; and the second in January 1987, when diseased plants showed the characteristic symptomatology, at the flowering stage.

Morphological studies

For morphological observations tissues were fixed in FAA (formaldehyde: AcOH:EtOH:H_2O; 30:5:50:15; v/v). For optical microscopy analysis adventitious and lateral roots were obtained by simple excision (Fig. 1).

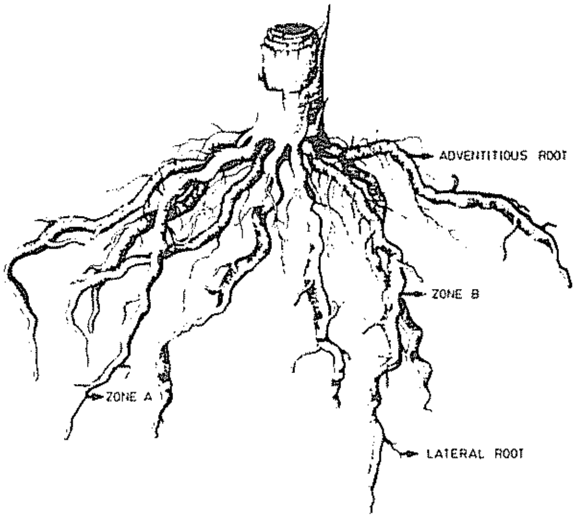

Fig. 1. *Zea mays* L. Diagram representing radical system exomorphology from an MRDV affected plant of maize.

In adventitious roots two zones were selected: apparently normal roots (Fig. 1; Zone A) and flattened roots (Fig. 1; Zone B), and then dehydrated in graded series of ethanol and ethanol:xylol mixture and embedded in paraffin. Serial 13-15 μm thick sections were obtained and stained using hematoxylin-safranin-fast green (11). The microtomic sections were observed in a C. Zeiss optical microscope.

Analysis of GA-like substances

Five grams of fresh weight (FW) roots were homogenized with 40 ml of 80% aqueous MeOH and liquid nitrogen in a mortar. The supernatant was removed and the residue re-extracted with an extra 20 ml of MeOH. The supernatants were pooled, filtrated and eluated through a reversed phase C_{18} column (13). After adjusting it to 50% MeOH by adding distilled water, the eluate was submitted to a second C_{18} column, then washed with an additional 20 ml 40% MeOH. The solvent was evaporated under vacuo and the remaining water freezed and freeze dried. The residue obtained contained free and conjugated froms of gibberellins.

SiO_2 partition column chromatography

The extract containing free and conjugated GAs was loaded onto a "short" SiO_2 partition column (13) by using 0.5 g of celite. The elution was first performed with a mixture of EtOAc:n-hexane (95:5) and then with 100% MeOH, in order to separate free, highly water soluble GAs from glucosyl conjugates and most of the highly water soluble very polar GAs (such as GA_{32}). Then, the free GA-fraction was evaporated under vacuo and chromatographied in a second SiO_2 column prepared as detailed by Durley *et al.* (7) and Powell and Tautvydas (22), the elution was carried out with a step-by-step 5% gradient of EtOAc (from 0 to 100%) in n-hexane (from 100 to 0%). Twenty four 10 ml fractions were collected, dried and tested on the dwarf rice cv. Tan-ginbozu bioassay (1/50 dilution). After this step, fractions were pooled again and gibberellins $\{1,2\ (n)\ -\ ^3H\}$ GA_1, specific activity 1.21 TBq $mmol^{-1}$ (Amersham) and $\{1.2\ (n)\ -\ ^3H\}$ GA_4, specific activity 1.4 TBq $mmol^{-1}$ (Amersham) were added as internal standards.

HPLC

Fractions coming from healthy roots were analyzed onto a Nucleosil $N(CH_3)_2$ - HPLC eluted with 99.9% MeOH in 0.1% AcOH for 50 min at a flow rate of 1 ml min^{-1}. For each of the 50 fractions collected, a bioassay was performed at 1/50 and 1/100 dilutions.

Due to the presence of more impurities, fractions coming from diseased roots were first subjected to reversed-phase C_{18} Z-module HPLC with the following solvent program: 0 to 30 min linear gradient from 37% to 100% MeOH in 0.1% AcOH, then 30 to 50 min 100% MeOH and at flow rate of 1 ml min^{-1}. Twenty five fractions of 1 min each (0 - 25 min), five fractions of 2 min each (26 - 35 min), and a bulk during 15 min more were collected and bioassayed (1/50 and 1/100 dilutions). Groups of bioactive fractions from both collection dates (December 1986 and January 1987) were pooled and injected onto a Nucleosil $N(CH_3)_2$ - HPLC eluted as above. Forty five fractions of 1 min and a bulk of 15 min more were collected and biossayed at the same dilutions.

Bioassay

To measure GA-activity, the dwarf rice (*Oryza sativa* cv. Tan-ginbozu) a microdrop assay (19) modified by using 0.5 μl application drop and measured after 48 h, was performed on each fraction eluted from SiO_2, C_{18} - HPLC and Nucleosil - HPLC columns. The biological activity was expressed as ng of GA_3 equivalents per g FW of maize tissues.

RESULTS

Macroscopic analysis

Macroscopic observations have shown that roots of infected plants were reduced compared with those of healthy plants, presenting flat zones and a decreased number of lateral roots which are shorter and esclerified. The radical system lost its typical aspect of "head of hair", characteristic of fibrous roots. Adventitious roots presented flattened zones and longitudinal ripping, nevertheless microscopy examination has not revealed necrosis or any kind of alterations.

Microscopy analysis

Adventitious root (Fig. 1; Zone A)

In Fig. 2 evident alterations were observed in the central cylinder; phloem poles presented their sieve-tube members partially or completely occluded by a light- brown unknown substance, which was deposited on the wall of sieve-elements confering to them a non characteristic thickness. Deposition of that substance advanced towards cell lumen. The infection began in phloem tissue, progressing to the xylem, endodermis and pericycle; cells constituting these tissues normally have thick and lignified walls; however, the grade of lignification is greater in affected roots than in healthy ones. Xylem showed vessels with reduced lumen, occupied by accumulation of an unknown material which dyed intense red. Xylem parenchyma was also affected; likewise, the parenchyma develops in relation to metaxylem elements, differentiating as vessels members (of varying size) replacing the parenchymatic cells.

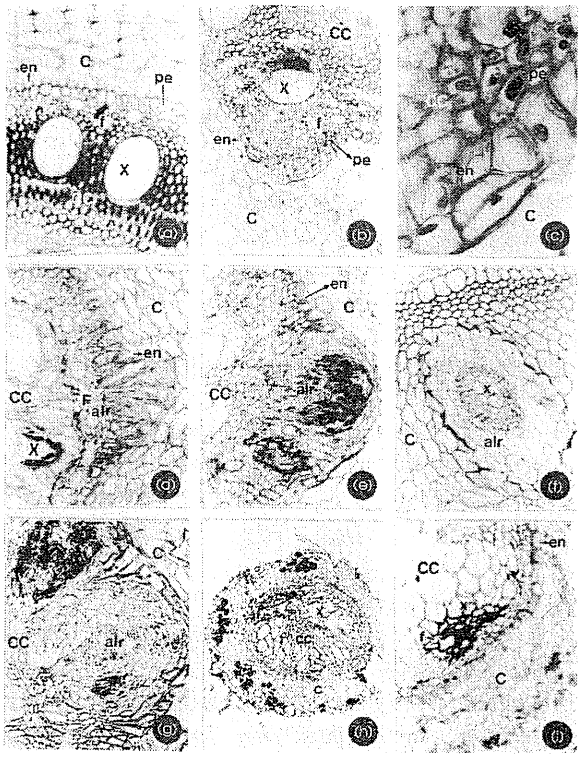

Fig. 2. *Zea mays* L. A — F Adventitious root transversal microtomic sections (Zone A, Fig. 1) showing in a) Alterations in central cylinder. B: Formation of abortive lateral root. C: Detail of radical primordia showed in b) with anomalous endodermis. d) Detail of thick and enlarged endodermis cells. e) Abortive lateral root with high grade of necrosis. f) Lateral root inmerse in the cortex of an adventitious root. g) Adventitious root transversal microtomic sections (Zone B, Fig. 1) showing evident disorganization in the lateral root primordia cells, which did not advance in its formation. h) Lateral root transversal microtomic sections indicating severe alterations in central cylinder. i) Detail of H presenting a phloem pole and xylem elements thick and partialy occluded.

Photographs a), b), e), g), h) and i) belongs sample collected on December/1986 and B, C, F to January/1987 a), b), d), e), f), g) and i) x 89; c) x 444 and L) x 36).

Abbreviations: alr, abortive lateral root. C, cortex. CC, central cylinder. en, endodermis. F, phloem. pe, percycle. X, xylem.

In all diseased adventitious roots we found formation of abortive lateral roots; these had a direct relation to previous alterations described in the central cylinder. Apparently, abortive roots (Fig. 2b) showed an initial normal development with predominant periclinal cell division in the pericycle and in the parenchyma cells belonging to the adjacent central cylinder zone. In a like manner, the endodermis began its cell division and, as is known, this layer intervenes in root primordia formation (2). As the orientation of cell divisions were disordered, endodermal tissue was not able to accompany primordia growth.

In some zones of the primordia (Fig. 2c), enlarged endodermis cells remained included, circled by other cells constituting the external limit of radical primordia. The endodermis showed excessive thickening of cell walls (Fig. 2d).

In healthy roots meristematic activity related to growth initiation of lateral roots was preceded by important changes in the de-lignification and remotion of secondary walls (2, 12). This phenomenon did not occur in deseased roots, so this zone either became necrotic (Fig. 2e), or the growth of the incipient root did not advance. In some cases, lateral roots were formed, reaching the cortex and remaining arrested in that zone (Fig. 2f); its disposition was parallel to the main axis of the adventitious root, taking place from the exodermis to the endodermis.

Adventitious root (Fig. 1; Zone B)

Alterations of adventitous roots were similar to the previous description; phloem poles were occluded and it was possible to distinguish enlargement of cells. The xylem, endodermis and pericycle showed the same alterations. Formation of abortive lateral roots which did not rise the cortex was frequent; an incorrect orientation of cell division was observed (Fig. 2g); the endodermis presented enlarged cells with thick walls rounding up the primordia. Disorganization of tissues advanced until general necrosis; some lateral roots emerged but were frequently abnormal, while others were normal and able to ramify.

Lateral root

In both Zone A and B (Fig. 1), infected plants presented some normal lateral roots with their central cylinder showing the typical polyarch structure; other lateral roots had an altered central cylinder, with the polyarch structure (Fig. 2h) impossible to identify. Several phloem poles were differentiated, although the xylem had an irregular aspect, with no poles and occupying almost all the space of the central cylinder. Thick and necrotic phloem poles, with vessels partially occluded by the same unknown substance present in adventitious roots, were also observed (Fig. 2i). Despite these alterations the presence of endodermis was identified, but there was no pericycle.

Analysis of gibberellins

Qualitative distribution of free GA-like substances in healthy and diseased maize roots assayed by dwarf rice (*Oryza sativa* L. cv. Tan-ginbozu) are summarized in Figs. 3, 4 and 5.

Roots of healthy plants collected in December 1986 (Fig. 3a) showed a very low level of free acid GAs. On the other hand, roots of diseased plants contained higher levels of these substances (Fig. 3b). A sharp peak eluted in fr 13 - 14 (Rt of authentic ^{3}H-GA$_1$ of C$_{18}$ - HPLC; a second peak running just before Rt of GA$_{20}$ was determined at min 18, and a lower bioactive zone was also present between min 23 - 26 (Rt of GA$_4$ and GA$_9$).

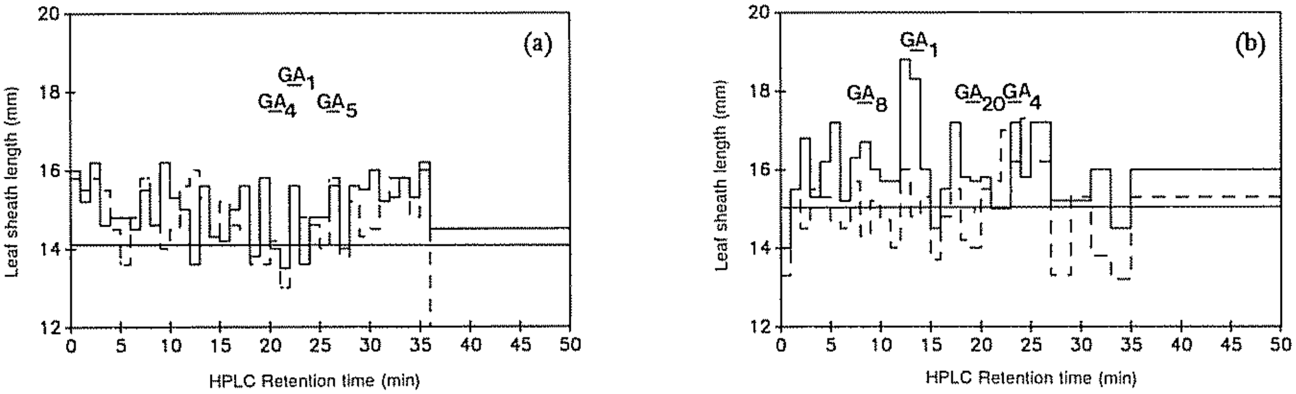

Fig. 3. a) Elution profile of free GA-like substances from healthy roots determined on Nucleosil-HPLC and bioassayed at 1/50 (solid line) and 1/100 (dashed line) dilutions. Collection December/1986. b) Elution profile of free GA-like substances from diseased roots determined on C$_{18}$ — HPLC and bioassayed at 1/50 (solid line) and 1/100 (dashed line) dilutions. Collec-tion December/1986.

Ordinate: Rice leaf sheath length (mm).
Abscissa: Fractions obtained from HPLC-chromato-graphy.

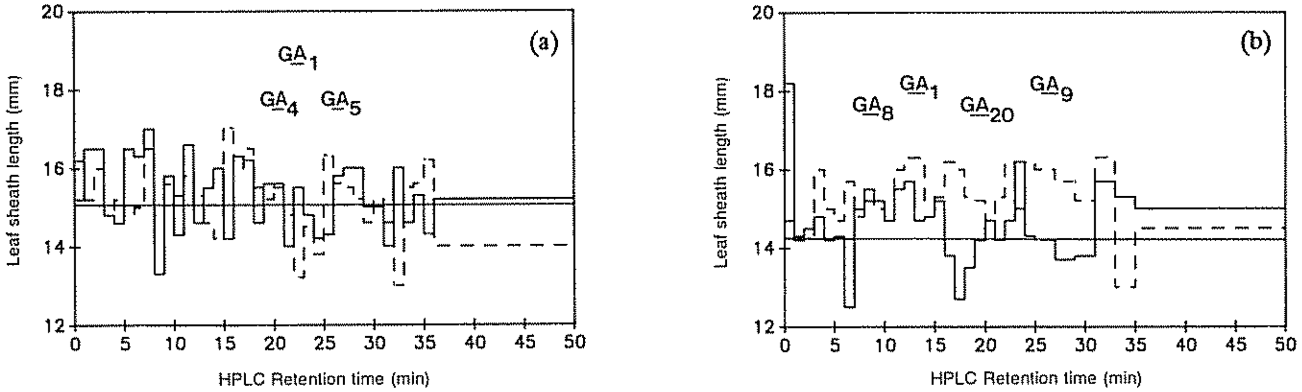

Fig. 4. a) Elution profile of free GA-like substances from healthy roots determined on Nucleosil-HPLC and bioassayed at 1/50 (solid line) and 1/100 (dashed line dilutions. Collection January/1987. b) Elution profile of free GA-like substances from diseased roots determined on C$_{18}$ — HPLC and bioassayed at 1/50 (solid line) and 1/100 (dashed line) dilutions. Collection January/1987.

Ordinate: Rice leaf sheath lenght (mm).
Abscissa: Fractions obtained from HPLC-chromato-graphy.

Roots collected in January 1987 did not show significant differences between healthy and diseased ones (Fig. 4a and 4b).

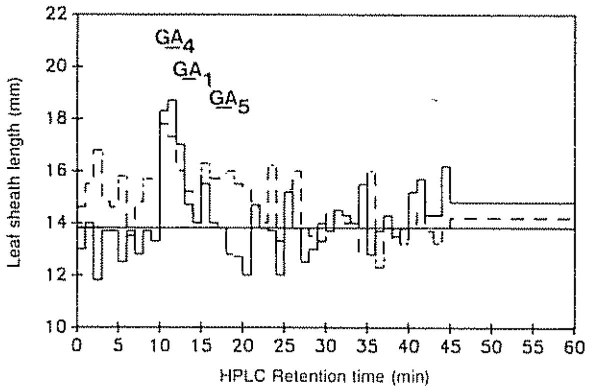

Fig. 5. Elution profile of free GA-like substances from diseased roots determined on Nucleosil-HPLC and bioassayed at 1/50 (solid line) and 1/100 (dashed line) dilutions. Both collection dates grouped (December/1986 and January/1987).

Ordinate: Rice leaf sheath length (mm).
Absissa: Fractions obtained from HPLC-ghromatography.

Figure 5 is a bioactive profile of grouped fractions from diseased roots, from collections in December 1986 and January 1987 analyzed on Nucleosil $N(CH_3)_2$ - HPLC. The same sharp peak of Fig. 3B eluting at Rt of GA_1 was found; minor active zones were also present.

DISCUSSION

Earlier, Castillo *et al.* (4) described the histological alterations caused by MRDV infection in basal nodes and internodes of maize shoots. These authors found that phloem and xylem tissues were those presenting more anomalies. Our studies on histopaphology of maize roots affected by "Mal de Río Cuarto" also showed dramatic alterations in their structure, mainly in the central cylinder.

MRDV-virus determined the presence of abnormal lateral roots as well as a great number of abortive lateral roots. There was a clear lignification of endodermis and central cylinder in diseased roots. All these modifications may reduce fibrous root volume and, occluded sieve-tubes members and xylem vessels may also disturb transport of nutrients, hormones and other metabolites from root to shoot.

With regard gibberellins, our results indicated that diseased roots collected in December 1986, when enations in leaves were evident, showed a different profile with enhanced levels of bioactivity of endogenous free GA-like substances, in comparison with that found for healthy roots.

The main region of GA-like activity (Fig. 3b) in diseased roots was associated with Rt of authentic ^3H-GA_1 standard. The reduced concentration of free GA-like substances in healthy roots may cause root growth and possibly internode elongation. This is in agreement with Tanimoto's (1987) results regarding the indispensable role played by GAs in root elongation at very low concentrations.

On the other hand, higher levels of GAs in diseased roots may imply that transport of free GAs to shoots or their catabolism might be prevented by virus attack.

The presence of biologically active GAs can be regulated by rate of biosynthesis including the metabolic conversion to an active form like GA_1.

Although a number of GAs have been identified in maize, Phinney (21) has postulated GA_1 as the only active GA in the elongation of *Gramineae* stem plants, especially in maize. Although our results might suggest a relationship between dwarfism in maize infected by MRDV and endogenous GA-level, quantification of endogenous GAs in healthy and diseased tissues, using more definitive methods (e.g. GC-SIM with stable-isotope-labeled internal standards) is required. The poor understanding of how virus-induced changes in host growth regulators causing alterations in growth, development and metabolism reflects the paucity of current knowledge of how endogenous regulators work in healthy plants. A greater understanding of the morphological and physiological basis of diseased plants will lead plant breeders to improve genotypics of maize in the search for more resistant varieties.

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