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# Influence of Vesicular-arbuscular Mycorrhiza on Phytohormone Balances in Maize (*Zea mays* L.)

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## Summary

The phytohormones abscisic acid (ABA), cytokinin (zeatin riboside) and indole-acetic-acid (IAA) were quantitatively determined in maize infected by the *Glomus* vesicular-arbuscular mycorrhizal (VAM) isolate T6 and in non-colonized controls. Measurements by indirect ELISA showed considerably higher levels of free ABA in VAM-infected than in control plants at all growth stages assayed (40, 60, 80, 110-day-old plants). In contrast, the amount of zeatin riboside was the same in infected and non-infected plants both for roots and shoots with the exception of the late plant growth phase (110-day-old plants), which showed an enhanced zeatin riboside level in VAM-colonized maize. Auxin (IAA) content, determined by a classical bioassay ($C_2H_4$-formation by pea epicotyl sections), was essentially the same in infected and non-infected roots. The concentrations of gibberellins could not accurately be determined by the release of $\alpha$-amylase from barley endosperm, since ABA from roots and shoots had overriding antagonistic effects in this assay. However, the test independently showed that the ABA-levels were higher in VAM-colonized roots of maize than in non-infected controls. As the plants had been grown under phosphate limitation, the increased levels of ABA in colonized roots could have resulted from a better $P_i$-supply acquired by the fungi or more likely from colonization of the roots by the fungi.

*Key words: Vesicular-arbuscular mycorrhiza, phytohormones in roots, abscisic acid, cytokinin, auxin.*

## Introduction

Most of the non-aquatic vascular plant species form vesicular-arbuscular mycorrhizal symbioses. Infection of a root by a VA-fungus can usually be assessed only by microscopic examination (Harley and Smith, 1983) or by detailed biochemical analysis (Schmitz et al., 1991). Some plants like maize or onion show a characteristic yellowing of their roots upon colonization, which is due to a still unidentified pigment (Becker and Gerdemann, 1977). Mycorrhizal plants generally have a better growth of roots and shoots (Gerdemann, 1964) and a more extensive branching of the root system, leading to substantial increases in the root surface area (Harley and Smith, 1983). Such plants are apparently more efficient in nutrient acquisition than non-infected controls. Factors reported to cause a better growth of VAM-infected plants are a better mobilization of minerals from soils such as $P_i$ (Harley and Smith, 1983), Zn and Cu (Kothari et al., 1991), an enhanced uptake of nitrogen (Barea et al., 1989), an increase in the transpirational flux and stomatal conductance (Harley and Smith, 1983; Kothari et al., 1990) and a reduction of manganese toxicity (Bethlenfalvay and Franson, 1989). It has been stated (Smith and Gianinazzi-Pearson, 1988; Schwab et al., 1991) that hormones are involved in establishing or regulating such growth changes. Information about this aspect is, however, scanty. Changes in the balances of all of the four major plant hormones (auxins, gibberellins, cytokinins and abscisic acid) have not yet been examined for any plant species and also not at different plant development stages in VAM-infected plants. In the present study, the levels of the two groups of hormones of most interest, ABA and cytokinins, have been determined in

VAM-infected maize and in non-infected controls using the ELISA-technique. The concentrations of auxins and gibberellins were measured by conventional bioassays.

## Materials and Methods

Maize (*Zea mays* L.), «Honeycomb» F-1 hybrid (from Altorfer Samen Comp., CH-Zürich) and the *Glomus* isolate T6 (kindly supplied by Dr. Dehne, Bayer AG, D-509 Leverkusen) were used for all the experiments. Plants were grown in 1.5 L plastic pots filled with 85 % expanded clay (Lecaton$^R$, 2–4 mm particle size, Leca D-2083 Halstenbek), 5 % soil («Einheitserde») and 10 % inoculum in a greenhouse at 20 °C and 70 % rel. air humidity. Experimental details and inoculum production were described previously (Schmitz et al., 1991). Pots with the control plants were filled with 95 % expanded clay and 5 % soil only. Watering was done daily, and Hoagland solution was supplied weekly without phosphorus and once a month with 1 mM $K_2HPO_4$. Plants were harvested 40, 60, 80 and 110 days after inoculation of the pots. Roots or shoots of 20 plants for each date were mixed and stored at −80 °C. Data in this work refer to g fresh weight of roots or shoots, respectively. The ratio between dry and fresh weights averaged 0.106 in roots and 0.176 in shoots of 110-day-old VAM-infected maize, and 0.085 in roots and 0.171 in shoots of the non-mycorrhizal controls of the same age. The ratio was 0.062 both in VAM infected and non-mycorrhizal roots of 60-day-old maize. The degree of mycorrhizal infection was determined by microscopic counting (Schmitz et al., 1991).

Purification steps for abscisic acid (adopted from Müller et al., 1989; Lehmann, 1988): 3–5 g (fresh weight) of roots or shoots were ground under liquid nitrogen and extracted overnight with 50 mL ice cold 80 % methanol supplemented with BHT (= butylated hydroxytoluene). The extract was filtered, supplemented with $^3$H-labelled abscisic acid (Amersham) as internal standard and diluted with $H_2O$ to a final methanol concentration of 70 %. Phenols were removed by shaking with 1 g PVP (polyvinylpyrrolidone) for 10 min. The extract was then passed through a SepPak $C_{18}$ column to remove lipids and most of the pigments. After the addition of 5 mL 100 % methanol and 10 mL 70 % methanol the fractions eluting from the column were evaporated to dryness at temperatures not exceeding 30 °C and dissolved in $H_2O$ at pH 2–3. The purification by the SepPak $C_{18}$ column was repeated exactly in the same way. ABA was then extracted with water-saturated diethylether and the organic fraction was evaporated to dryness, dissolved in $H_2O$ (pH 4.0) and stored at −20 °C prior to use. The yield in % was 73 ± 8 (n = 40).

Extraction of cytokinins (adopted from Müller et al., 1989; Köhler, 1988): 3–5 g (fresh weight) were ground and extracted overnight with 50 mL 70 % cold methanol supplemented with BHT and filtered. After the addition of $^3$H-labelled dihydrozeatin and treatment with PVP, cytokinins were passed twice through a SepPak C-18 column as done for ABA. Fractions eluting were evaporated to dryness, dissolved in 50 mM $NaHCO_3$, pH 9.3, adjusted to pH 7–8 and extracted with water-saturated butanol. The organic phase was evaporated to dryness at 40 °C, dissolved in $H_2O$ and stored at −20 °C. The yield in % was 63 ± 5 (n = 40).

Determination of ABA and cytokinin concentrations by the indirect ELISA assay: Polyclonal antibodies against ABA and cytokinins were raised as described by Marx et al. (1988), and the protocols for the preparation of the ABA-turkey ovalbumin- and the zeatin riboside-bovine albumin-conjugates were taken from Weiler (1979, 1980). The quantitative determinations of ABA and zeatin riboside by the indirect ELISA using biotinylated anti-rabbit antibodies and streptavidin biotinylated peroxidase (Amersham, Braunschweig) are standard procedures (see Dai et al., 1992). The polyclonal antibodies raised against ABA showed some cross-reactivity to glucopyranosyl abscisate (20 %) and those against zeatin riboside to zeatin (60 %), dihydrozeatin (60 %), adenosine (0.26 %) and ribose (0.01 %).

Bioassays for determining gibberellin and auxin concentrations in VAM-infected maize and in controls: The concentration of gibberellins in roots and shoots was measured by the production of α-amylase in barley endosperm (Jones and Varner, 1967) as described earlier (Zimmer and Bothe, 1988). Auxin concentration was determined by the formation of $C_2H_4$ by pea epicotyl segments (Lieberman and Kunishi, 1975), as reported previously (Zimmer et al., 1988). Measurements in crude extracts of roots or shoots gave no accurate data for both phytohormones; therefore, a partial purification was necessary. For this, 9 g fresh weight of either roots or shoots was ground in liquid nitrogen and extracted overnight with 100 mL 70 % methanol at 4 °C in the presence of BHT. After filtration and evaporation to dryness, phytohormones were redissolved in 2 mL $H_2O$, adjusted to pH 7–8 and extracted 3 times with ethylacetate. The ethylacetate fraction was evaporated to dryness and resuspended in 2 mL $H_2O$. An aliquot of 0.2 mL (1 : 10 diluted) was assayed for gibberellin content using 10 barley seed halves (embryos removed) for each test. Another 0.1 mL of the same fraction (also diluted 1 : 10) was taken for the auxin assay ($C_2H_4$-formation by 12 pea epicotyl segments, see Zimmer et al., 1988). The average recovery in this enrichment procedure in % was 71 ± 10 (n = 6) for indoleacetic acid, whereas that for gibberellin could not be determined accurately (see Results).

## Results

### *a) Characterization of the plant material*

Different investigators (see Smith and Gianinazzi-Pearson, 1988; Harley and Smith, 1983) agree that mycorrhizal colonization stimulates growth of crops, but the extent of this growth stimulation is somewhat variable and depends on the nutritional status of the plants, particularly their supply of phosphorus. A characterization of the plants used for the present study is, therefore, necessary (Table 1). Maize used for the ELISA-tests was colonized more than 60 % by VAM (determined by microscopic counting) after 40 days of growth, which increased to about 80 % at the end of the vegetation period. At all four stages assayed (40, 60, 80, 110 days), VAM infected plants had much higher root and shoot weights compared with the controls, whereas the ratio between shoot and root weights was more or less the same. VAM colonized maize had broader and longer leaves of dark

Table 1: Changes in weight during the growing period in VAM-infected corn and in control plants.

| age of plants | 40 | | 60 | | 80 | | 110 | |
|---|---|---|---|---|---|---|---|---|
| developmental stage | juvenil | | shooting | | | | mature | |
| | 4–5 leaves | | 8–10 leaves | | ear development | | grain filling | |
| degree of mycorrhization % | C | M | C | M | C | M | C | M |
| total | 7 | 63 | 1 | 76 | 2 | 76 | 5 | 81 |
| vesicles | | 51 | | 59 | | 68 | | 70 |
| arbuscules | | 37 | | 42 | | 45 | | 37 |
| fresh weight/plant (g) | | | | | | | | |
| shoot | 3.0 | 4.0 | 11.9 | 21.0 | 29.5 | 34.7 | 29.3 | 35.9 |
| roots | 4.0 | 5.3 | 5.1 | 9.7 | 12.1 | 16.2 | 11.9 | 16.4 |

The percentage of mycorrhizal colonization was determined by microscopic counting. M = mycorrhizal plants colonized with *Glomus* T6, C = control without VAM. The low values determined in the controls are due to cross-infections.

Table 2: The content of abscisic acids (ABA) in mycorrhized and non-mycorrhized corn.

| age of plants (d) | 40 | | | 60 | | | 80 | | | 110 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | M | Δ | C | M | Δ | C | M | Δ | C | M | Δ |
| degree of mycorrhization (%) | 7 | 63 | | 1 | 76 | | 2 | 76 | | 5 | 81 | |
| roots | 35 | 39 | 4 | 53 | 86 | 33 | 48 | 98 | 50 | 60 | 100 | 40 |
| | 44 | 63 | 19 | 65 | 130 | 65 | 57 | 178 | 121 | 64 | 73 | 9 |
| | 40 | 66 | 26 | 41 | 89 | 48 | 53 | 111 | 58 | 76 | 149 | 73 |
| | | | | 33 | 57 | 24 | 59 | 88 | 29 | 44 | 60 | 16 |
| | | | | | | | | | | 51 | 145 | 94 |
| | | | | | | | | | | 21 | 60 | 39 |
| | | | | | | | | | | 24 | 73 | 49 |
| | | | | | | | | | | 25 | 64 | 39 |
| $\bar{x}$ (Δ) | | | 16±11 | | | 43±18 | | | 65±40 | | | 45±26 |
| shoots | 49 | 127 | 78 | 72 | 87 | 15 | 45 | 103 | 58 | 70 | 136 | 66 |
| | 53 | 123 | 70 | 40 | 68 | 28 | 45 | 106 | 61 | 50 | 118 | 68 |
| | | | | | | | | | | 91 | 132 | 43 |
| $\bar{x}$ (Δ) | | | 74± 6 | | | 22± 9 | | | 60± 2 | | | 59±14 |

The concentrations of ABA were determined by the indirect ELISA-assay after partial purification of the extracts (see Materials and Methods). Data are given pmol ABA/g fresh weight.
(Δ): difference; $\bar{x}$: means.

greenish appearance. Roots exhibited their characteristic yellow colour, were thinner but had extensive branching with an increase in the surface area. Inevitably, the controls also showed some minor colonization, probably by pathogenic fungi with this material grown in the greenhouse, despite of sterilization of the soil and expanded clay prior to inoculum. Differences in all parameters between VAM-infected and control plants were, however, clear-cut (Table 1).

*b) Determination of the ABA and cytokinin contents*

The concentrations of ABA and cytokinins could not be determined accurately in crude extracts by indirect ELISA, because no parellelity was obtained when the extinctions (after log. transformation) were plotted against different amounts of crude extract or authentic phytohormones. Since this was presumably due to unspecific adsorption of antibodies to proteins, partial purification of ABA and cytokinins from crude extracts was mandatory. With each of the preparations assayed, it was necessary to compare different concentrations of partially enriched phytohormones with a standard curve in the ELISA to ensure the parallelity of the curves. In addition, the recovery of the two phytohormones varied from one enrichment to the next to some extent. For calibration, the extract was, therefore, supplemented with an internal radioactive standard prior to the start of the enrichment procedure. The concentrations of ABA in VAM-infected and in control plants were always determined side by side, and each of the two figures for one age in Tables 2 and 3 represents the labour of approximately 1 week. The values, although somewhat variable, unambiguously showed that the concentration of free ABA in roots and shoots was always higher in infected plants (Table 2). This was true for all four developmental stages investigated. The differences in the concentration of ABA between VAM-infected plants and

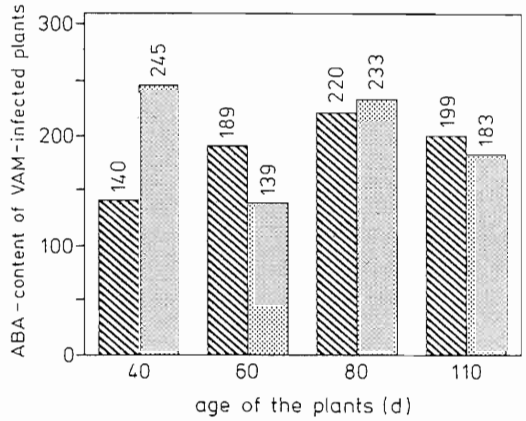


Fig. 1: The enhancement of ABA-content in VAM-infected maize compared with control plants. The ABA content of roots or shoots of non-infected plants was set to 100%. The data of Table 2 were used. For experimental details see Materials and Methods. ▨ roots, ▩ shoots.

controls was particularly pronounced after 80 days, both in roots and shoots (Fig. 1). The amount of conjugated ABA was not determined as the polyclonal antibodies only weakly reacted with glucosylated abscisate, and as conjugated phytohormones were found to be morphogenetically inactive or less active (Letham and Palni, 1983; Moore, 1989). In contrast, the concentration of zeatin riboside (plus zeatin and dihydrozeatin) also determined by ELISA was not different when 40-, 60-, 80-day-old infected and control plants were compared (Table 3). A difference was, however, observed at the late growth phase (110 days) where mycorrhizal plants showed higher levels of this phytohormone in the roots.

Table 3: The content of cytokinins in mycorrhized and non-mycorrhized corn.

| age of plants (d) | 40 | | 60 | | 80 | | 110 | |
|---|---|---|---|---|---|---|---|---|
| | C | M | C | M | C | M | C | M |
| degree of mycorrhization | 7 | 63 | 1 | 76 | 2 | 76 | 5 | 81 |
| cytokinin content (pmol zeatin riboside/g fresh weight) | | | | | | | | |
| roots | 8.0 | 7.5 | 8.0 | 7.4 | 4.0 | 4.0 | 15 | 29 |
| | 10.6 | 6.9 | 6.0 | 12.0 | 4.0 | 2.2 | 15 | 27 |
| | 2.0 | 7.0 | 6.0 | 7.0 | 3.0 | 3.0 | 13 | 38 |
| | 3.0 | 9.0 | 11.0 | 9.0 | 1.0 | 5.0 | 8 | 51 |
| | 11.0 | 16.0 | 5.0 | 6.0 | 4.7 | 3.7 | 11 | 22 |
| | | | | | | | 8 | 26 |
| | | | | | | | 7 | 25 |
| $\bar{x}$ | 6.9±4.2 | 9.3±3.8 | 7.2±2.4 | 8.3±2.3 | 3.3±1.4 | 3.6±1.1 | 11± 3.4 | 31±10.1 |
| shoots | 2.9 | 2.4 | 7.0 | 10.0 | 5.0 | 2.2 | 27 | 84 |
| | 2.1 | 1.0 | 9.0 | 9.0 | 5.0 | 2.4 | 92 | 104 |
| | 2.0 | 2.2 | 9.7 | 8.4 | 6.0 | 8.0 | 59 | 59 |
| | | | 7.5 | 6.0 | | | | |
| $\bar{x}$ | 2.3±0.5 | 1.9±0.7 | 8.3±1.3 | 8.4±1.7 | 5.3±0.6 | 4.2±3.3 | 59±33 | 82±23 |

The content of zeatin riboside was determined by the indirect ELISA-assay after partial purification of the extracts (see Materials and Methods). Data are given in pmol zeatin riboside/g fresh weight. For the specificity of the polyclonal antibodies used see Materials and Methods.

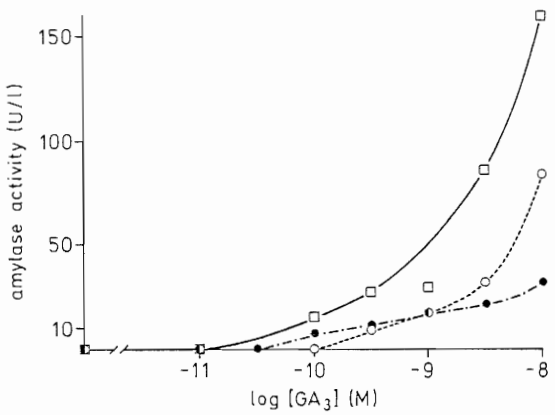


Fig. 2: The release of α-amylase activity from barley endosperm in dependence of gibberellins and extracts from roots of infected and non-infected maize. The determination of α-amylase activity is based on the release of p-nitrophenol from p-nitrophenyl-α-maltoheptaside and is given in units of enzyme activity/l (see Zimmer and Bothe, 1988). □——□ control curve with authentic $GA_3$; ○- - - -○ same concentrations as before, however, flasks supplemented with partially purified extract from non-infected roots, 900 mg fresh weight prior to extraction; ●- - - -● same concentrations as before, however with extracts from infected (54 % infection) roots, 900 mg fresh weight.

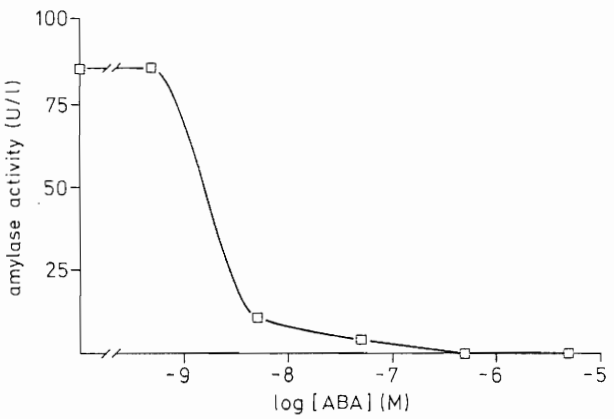


Fig. 3: The inhibition of the $GA_3$-stimulated release of α-amylase activity by ABA. The experiment was performed with authentic $GA_3$ (= $10^{-5}$ M in the assay) and authentic ABA. For experimental details see Zimmer and Bothe, 1988.

*c) Assay for gibberellins*

It was attempted to determine the concentration of gibberellins by the release of α-amylase from barley endosperm. Figure 2 represents a typical dose response curve for authentic $GA_3$. The addition of partially purified extract, surprisingly, did not stimulate α-amylase activity neither in the absence of external $GA_3$ nor at any of the $GA_3$ concentrations assayed. On the contrary, a marked inhibition could consistently be observed, which was particularly distinct with the extract from mycorrhizal roots at different concentrations of external $GA_3$ (Fig. 2). This inhibition was apparently due to ABA in this extract. A control experiment with authentic ABA (Fig. 3) indicated that this phytohormone antagonistically affected the $GA_3$-dependent release of α-amylase. Thus, the data of Fig. 2 obtained with this classical bioassay independently confirmed the results from the ELISA-measurements that the concentration of ABA was significantly higher in VAM-colonized roots than in the controls. As the effects of ABA in extracts were overriding, the test could not give estimates for the concentrations of gibberellins in roots. However, the levels of GA in mycorrhizal

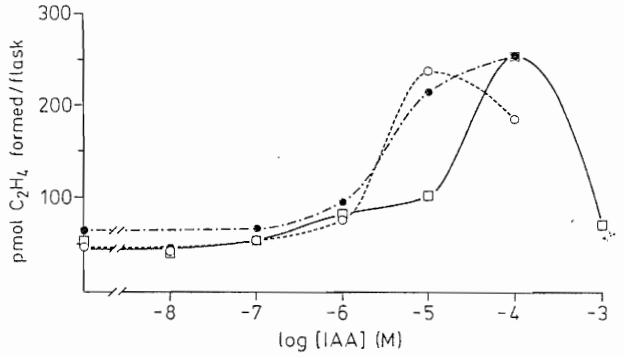


Fig. 4: $C_2H_4$-formation by pea epicotyl segments in dependence of indole acetic acid (IAA) and extracts from roots of infected and non-infected maize. For experimental details see Zimmer et al., 1988 and Materials and Methods. □——□ control curve with authentic IAA; O·····O same concentrations as before, however, flasks supplemented with partially purified extract from non-infected roots, 450 mg fresh weight before extraction; ●·····● same concentrations as before, flasks now supplemented with partially purified extract from infected roots, 450 mg fresh weight before extraction.

Table 4: Influence of the amount of root extract on the $C_2H_4$-formation by pea epicotyl sections.

| amount of root extract (mg/flask) | maize without VAM | controls | maize with VAM |
|---|---|---|---|
| | (pmol $C_2H_4$ formed/flask) | | |
| – extract | | 48 | |
| 90 | 50 | | 51 |
| 225 | 52 | | 51 |
| 450 | 61 | | 65 |
| 900 | 58 | | 62 |
| 2250 | 51 | | 50 |
| + IAA alone ($10^{-4}$ M) | | 255 | |

The root material used for extraction was taken from 110-day-old plants. Infection: 0 % for the controls and 54 % for the infected material. The amount of root extract refers to the starting material (in mg fresh weight) used for the extraction prior to the enrichment by ethylacetate. For experimental details see Materials and Methods.

and non-mycorrhizal roots unlikely were largely different, because otherwise a differential effect should have been seen in this assay.

*d) Determination of the auxin content*

$C_2H_4$-formation by pea epicotyl sections is stimulated by auxin (Lieberman and Kunishi, 1975) and provides a simple and sensitive assay for this phytohormone. Extracts from VAM-infected and non-mycorrhizal roots stimulated $C_2H_4$-formation to the same extent. However, maximal stimulation by the extracts was less than 10 % of the control, with IAA of higher extract concentrations being inhibitory (Table 4). A dose response curve (Fig. 4) showed that the optimal concentration of IAA (without extract) was $10^{-4}$ M. When the different concentrations of IAA were supplemented with partially purified extract from roots (at the concentrations that stimulated maximally, see Table 4), the optimal concentration of IAA shifted towards $10^{-5}$ M with both root types (Fig. 4). Thus, the extract contained component(s) that enhanced the sensitivity of the assay to IAA. An interference by methionine is feasible, since methionine dependent formation of $C_2H_4$ in plants is known to be stimulated by auxin. Methionine was, however, not the component that interferred in $C_2H_4$-formation by pea epicotyl sections because this amino acid when assayed alone was completely inactive in the assay, and its presence did not alter the dose response curve for auxin (not documented).

Table 5: Determination of the IAA-content in VAM-infected and non-infected maize roots.

| root material | $C_2H_4$ formed (pmol/flask) | | | | |
|---|---|---|---|---|---|
| | control without extract | with extract | with extract + IAA (after extraction) | with extract + IAA (before extraction) | IAA equivalents in the root* (nmol/g FW) |
| 60-day-old maize: | | | | | |
| + VAM | 68 | 91 | 214 | 201 | 5.6 ± 1.4 |
| – VAM | 68 | 86 | 230 | 198 | 4.0 ± 1.6 |
| 110-day-old maize: | | | | | |
| + VAM | 80 | 102 | 363 | 283 | 3.0 ± 0.8 |
| – VAM | 76 | 110 | 441 | 337 | 3.1 ± 1.6 |
| + VAM hydrolysed | 75 | 135 | 477 | 343 | 6.5 ± 2.9 |
| – VAM hydrolysed | 75 | 140 | 576 | 433 | 5.5 ± 3.2 |

The infection degree of the material used was: 60-day-old plants = 54 %; controls = 0.6 %; 110-day-old plants = 55 % and controls = 0 %. Extract was always prepared from 450 mg fresh weight of roots. Enrichment of IAA in the extract was performed by the ethylacetate extraction procedure. Where mentioned, the root extract was hydrolyzed for 1 h with 1 N NaOH at room temperature prior to the ethylacetate extraction and assay. The test system contained in 1 mL final vol: 0.1 mL root extract where indicated 10 nmol indole acetic acid, and incubation buffer. For other conditions of assay (the $C_2H_4$-formation by pea epicotyl sections) see Materials and Methods. * The IAA equivalents in the roots were corrected by the average recovery given by the term [(4)-(2)]/[(3)-(2)].

Keeping in mind that extracts shifted the optimal requirement for IAA in this bioassay to a lower concentration, levels of IAA in maize roots could be estimated (Table 5). In both colonized and non-infected roots the concentrations of free IAA were around 3 nmol/g fresh weight, and this amount increased to approximately 6.0 nmol when the extracts were subjected to hydrolysis with NaOH prior to the enrichment procedure and assay. Thus, the levels of free and conjugated IAA were not significantly affected either by the age of the plants or by mycorrhizal colonization (Table 5).

## Discussion

The present investigation of the changes in phytohormone balances affected by VA-mycorrhizal colonization revealed positive results in the case of ABA. In all of the many samples assayed, the concentration of this phytohormone both in shoots and roots was always higher in VAM-infected plants than in the controls. This result was seen in the ELISA-tests as well as – somewhat unexpectedly to us – in the bioassay for gibberellins (release of α-amylase activity) where the inhibitory concentrations of ABA were higher in mycorrhizal than in non-infected roots. It cannot be argued

that this increase in the ABA level simply reflects the better growth of the plants, since the differences were observed at all four plant development stages analyzed. In addition, only the level of ABA changed, whereas those of cytokinins at the first three stages (40, 60, 80 days) and of auxins remained unaltered. With the other phytohormones, any variation in the content of auxin was not expected, and the present results do not allow any firm conclusion about gibberellins due to the test system used. Cytokinin content was significantly higher in mycorrhizal roots at the end of the vegetation period (110-day-old plants). This result should be interpreted with some caution, because particularly the control plants may not have been optimally supplied with nutrients at the end of their vegetation period in the pots and under greenhouse conditions. The control plants, 110 days of age, also had an enhanced cytokinin level.

In order to get high infection rates and an effect of mycorrhizal colonization on the plant growth, the plants were grown under non-severe $P_i$-limitation. With saturating levels of $P_i$, maize plants were colonized by less than 20% with fungi, and VAM-infected plants did not show an improved growth (not documented). It cannot be ruled out that the fungi were metabolically inactive under these conditions. In contrast, under the $P_i$-limitations of the present study, the increase in ABA content could have been due to an improved $P_i$ status in the plants acquired by the fungi. It is, however, more likely to us that the effects were caused by the fungal colonization itself.

As VAM-fungi cannot be grown independently of the host, the relative contributions of fungus and plant to the enhanced ABA-production are unknown. Many microorganisms, e.g. *Azotobacter* (Taller and Wong, 1989) or *Rhizobium* (Sturtevant and Taller, 1989), are known to produce phytohormones, and mycorrhizal fungi might also do so. This was shown for mycelia of some non-VAM fungi (Crafts and Miller, 1974) and also for germinated spores of *Glomus mosseae*, which synthesized cytokinin and gibberellin-like stubstances (Barea and Azcón-Aguilar, 1982). To our knowledge, production of ABA by VAM fungi has not unambiguously been shown as yet. In the symbiosis, enhanced cytokinin levels resulting from mycorrhizal infection were described to occur in the rangeland grass (*Bouteloua gracilis*, Allen et al., 1980) whereas the total activity of GA-like components increased in leaves but decreased in roots (Allen et al., 1982). A component with ABA-like properties was present but could not be identified in the latter study. It has also been described that VAM colonization increased the flux of cytokinins from the roots to the shoots in *Citrus* (Dixon et al., 1988). In contrast, Baas and Kuiper (1989) described for *Plantago major* that addition of phosphorus increased cytokinin levels more than VAM infection did and concluded that cytokinins are not the primary growth substances involved in mediating VAM-effects. Cytokinins typically stimulate protein and chlorophyll synthesis as well as cell division and expansion in plants (van Staden and Davey, 1979), but are generally not involved in the regulation of metabolite fluxes from one organ to the other. Thus, in line with Baas and Kuiper (1989), a specific regulatory role in the symbiosis between fungi and plants is difficult for us to envisage for cytokinins.

ABA, on the other hand, is the typical stress hormone. The levels of ABA in plants increase in response to conditions such as drought (Lachno and Baker, 1986), flooding (Zhang and Davies, 1987) and salinity (Amzallag et al., 1990; Kefu et al., 1991). These increases are generally observed within hours after the onset of the stress, whereas long-term effects of ABA could be involved in the regulation of the plant-mycorrhizal symbiosis. ABA regulates transpirational fluxes and stomatal closures (Raschke, 1975) as well as allocation of nutrients from and to the shoots and of organic carbon vice versa (Schwab et al., 1991). In mycorrhizal plants the transpirational rates were found to be 30% higher than in the controls (Kothari et al., 1990). ABA as well as auxins (Pilet and Saugy, 1987) and cytokinins (Stenlid, 1982) were shown to inhibit elongation of slow growing roots, whereas a promotion of the elongation of fast growing roots was affected specifically by ABA (Pilet and Saugy, 1987; Mulkey et al., 1983). From all this, a close correlation between the effects caused by ABA and by mycorrhizal colonization is conceivable. However, even for the non-infected plants, it is almost unknown which cells within a plant and which compartment within a cell are touched by ABA. The interactions between mycorrhizal fungi and plants are even more complex, and clearly many other factors besides ABA are involved in regulating this symbiosis.

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