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Mycorrhiza

Presence of specific polypeptides in onion roots colonized by *Glomus mosseae*

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Abstract. We studied changes in gene expression during the establishment of vesicular-arbuscular (VA) mycorrhizal symbiosis. Polypeptides were obtained by in vitro translation of total root RNA extracted from VA-colonized and noncolonized root-tissue of onion (*Allium cepa* L. cv. Babosa), and resolved by two-dimensional polyacrylamide gel electrophoresis. VA mycorrhization led to a specific appearance of eight new polypeptides, and the disappearance of seven polypeptides in VA-colonized root. Our findings indicate that gene expression is altered in response to morphological and physiological changes resulting from the establishment of VA mycorrhizas.

Key words: *Allium cepa* – Gene expression – *Glomus mosseae* – VA mycorrhizas – Specific polypeptides

Introduction

Vesicular-arbuscular (VA) mycorrhizas, like other mutualistic symbioses between plants and microorganisms, are biotrophic entities in which the heterotrophic partner is supplied with carbon compounds from the autotrophic host, while the hyphae enhance the uptake of mineral nutrients by the plant (Harley and Smith 1983). The colonization of plant roots by VA mycorrhizal fungi involves the formation of intercellular hyphae – highly branched intracellular arbuscules and vesicles scattered throughout the root (Bonfante-Fasolo 1984). The complexity of the life cycle of the fungus within the plant indicates that the VA mycorrhizal symbiosis cannot be the result of the expression of a unique gene (Anderson 1988). It is likely that the formation of a symbiotic organ is basically controlled by tightly coordinated "symbiotic genes" whose expression leads to specific and non-specific signals recognized by both partners.

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We report here a distinctive pattern of gene expression in roots colonized by the VA mycorrhizal fungus *Glomus mosseae* observed by in vitro translation of total root RNA followed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE).

Materials and methods

Plants and growth conditions

Onion plants (*Allium cepa* cv. Babosa) were grown in 50-ml pots of soil collected from the province of Granada, Spain. The soil, a "reddish-brown" type with pH 7.6 (for full details see Garcia-Romera and Ocampo 1988), was steam-sterilized and mixed with sterilized quartz sand in the proportions 1:9 (v:v). Seeds were surface-sterilized with 2% $HgCl_2$ for 10 min and thoroughly rinsed with sterile water. After germination, seedlings were selected for uniformity before planting. In each pot, 200 surface-sterilized spores (MacDonald 1981) of *G. mosseae* (Nicol. & Gerd.) Gerd. and Trappe were placed a few millimetres under the seedlings. Uninoculated control pots were also sown. Plants were grown in a chamber with light from Silvania incandescent and cool-white lamps, 400 nmol $m^{-2} s^{-1}$, 400–700 nm, with a 16–8 h light-dark cycle at 25–19° C and 50% relative humidity. Plants were watered from below using a capillary system and fed with a nutrient solution (Hewitt 1952) lacking phosphate for VA-inoculated plants.

Mycorrhizal measurements

Plants were harvested after 60 days. Parts of the root system were cleared and stained (Phillips and Hayman 1970) and the percentage of VA root-length colonization was measured by the gridline intersect method (Giovannetti and Mosse 1980).

RNA isolation and in vitro translation

Total RNA was isolated from 2 g of root tissue by LiCl precipitation and phenol-chloroform extraction as described by Cathala et al. (1983). The RNA (8 µg/reaction) was translated in an RNA-dependent, nuclease-treated, rabbit reticulocyte lysate system according to the the manufacturer's instructions (Boehringer Mannheim) with 20 µCi of ^{35}S-methionine (1000 Ci/mmol, Amersham) per reaction.

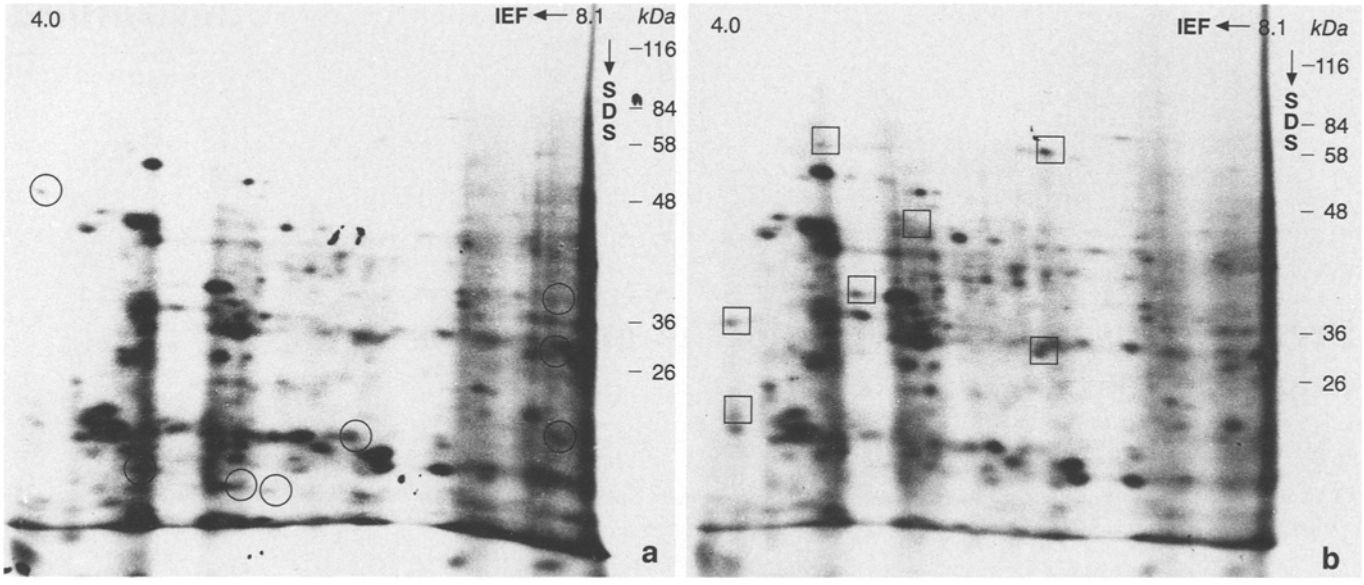

Fig. 1. Fluorograms of two-dimensional gels of in vitro translation products from total RNA extracted from VA mycorrhizal (**a**) and nonmycorrhizal (**b**) onion roots. The appearance (*open circles*) and disappearance (*open squares*) of polypeptides in VA mycorrhizal tissue are labeled

Two-dimensional electrophoresis

The translation products were separated by 2-D PAGE (O'Farrell 1975) using a 12.5% polyacrylamide slab gel for the second dimension. The first dimension isoelectric focusing contained 2% ampholines (pH 3–10) and 10.5% ampholines (pH 5–7). Samples were always placed on the basic end of the focusing gel. Prestained molecular weight and isoelectric focusing standards from Sigma were used. Usually between 3×10^5 and 10^6 cpm incorporated into translation products was loaded into the first dimension gel. After the second dimension was run, the gels were fluorographed with Amplify (Amersham) for 30 min, dried and exposed to X-OMAT film (Kodak) for 6 days at −70° C.

Results

Microscopic observations of stained roots showed no fungi in uninoculated controls and 45% of root length VA colonized in inoculated plants (data not shown). The experiments were repeated three times with similar results. The application to the first dimension of 10^6 cpm incorporated into translation products was most effective in visualizing the different polypeptides.

Comparison of the fluorograms of VA mycorrhizal onion roots with nonmycorrhizal root two-dimensional patterns (Fig. 1a, b) indicated that two major changes in polypeptide synthesis occurred in VA mycorrhizal plants. In VA mycorrhizal tissues, eight specific polypeptides appeared (Fig. 1a), with apparent molecular masses of 56, 38, 28, 18, 17, 15, 14 and 13 kDa and isoelectric points of 4.6, 7.5, 7.5, 8.0, 6.3, 5.0, 5.6 and 5.6, respectively. Seven root polypeptides with molecular masses of 70, 67, 46, 41, 37, 30, and 20 kDa and isoelectric points of 5.1, 6.4, 5.6, 5.3, 4.4, 6.3 and 4.3, respectively, disappeared (Fig. 1b). Interestingly, of the eight new polypeptides in the VA mycorrhizal roots, six were of low molecular weight (13 to 28 kDa). In contrast, of the seven polypeptides which disappeared from these roots, six had molecular masses ranging from 30 to 67 kDa.

Discussion

Little is known about the molecular mechanisms by which genetic factors exert their influence on VA mycorrhizal development in plant roots. Our results indicate extensive changes in gene expression in VA mycorrhizal root tissue in response to morphological and physiological changes resulting from the establishment of VA mycorrhiza, with the accumulation of mycorrhiza-specific polypeptides. The existence of symbiotic specific polypeptides in VA mycorrhizal roots was first suggested in 1990 by Wyss et al. By in vitro translation of total root RNA and 2-D PAGE, we were able to demonstrate that at least 15 different messages were altered due to VA colonization. The new polypeptides observed in VA mycorrhizal roots may be of fungal or of plant origin. Changes at the level of gene expression associated with this symbiosis are difficult to study because fungi that form VA mycorrhizas have not yet been cultured axenically in the absence of plant roots. We also observed a reduction in the expression of certain root genes in VA mycorrhizal roots. Reductions in gene expression have also been observed in other mutualistic associations such as *Rhizobium*-legume (Lullien et al. 1987; Gloudemans et al. 1989) and *Pisolithus–Eucaliptus* (Hilbert and Martin 1988) symbioses, but their role remains unknown.

Our analyses by 2-D PAGE of the in vitro translation products of total root RNA were carried out using an established VA mycorrhizal colonization, in which the

fungus was at the end of its logarithmic stage of growth (data not shown). The possibility that different patterns of gene expression appear during different stages of root colonization by VA mycorrhizal fungi cannot be ruled out. Further studies with purified mRNA prepared from free-living partners and endomycorrhizal tissues during the process of VA colonization should clarify the regulatory mechanisms involved.

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