Phenols in mycorrhizal roots of *Arachis hypogaea*

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Summary. Roots of mycorrhizal groudnut plants had higher amounts of phenols compared to non-mycorrhizal roots. Histochemical study of the mycorrhizal root revealed the accumulation of phenols in hyphae and arbuscules of the fungus within the host.

Enhanced plant growth due to vesicular-arbuscular (VA) mycorrhiza has been commonly attributed to increased nutrient uptake. However, recent studies suggest that VA mycorrhizal infection may change the biochemical composition of the host plant. Differences in sugar[2], lipid[3,4], amino acid[5], and steroid[6] composition between mycorrhizal and non-mycorrhizal plants have been observed. There is only one report on increasing phenol synthesis of the host following VA mycorrhizal inoculation[7]. The present study compares the phenolics in mycorrhizal and non-mycorrhizal groundnut plants.

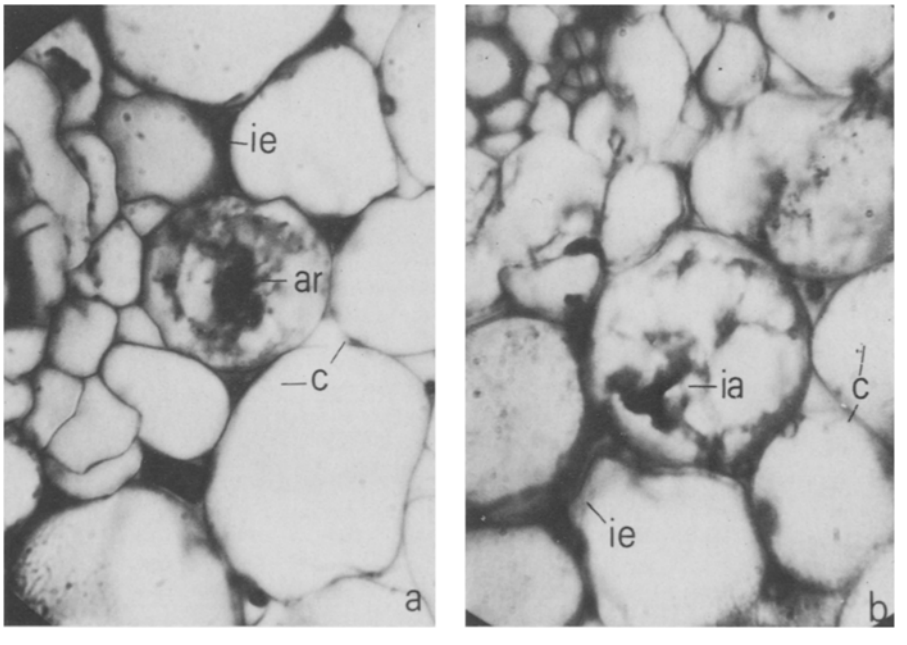
Methods. Groundnut (*Arachis hypogaea* L cv DH3-30) was grown in 15-cm pots filled with 3 kg of steam-sterilized soil with and without mycorrhizal inoculum. The soil used was a red sandy loam of pH 5.5, deficient in P (3 mg available P per kg soil extractable with $NH_4F + HCl$). Mycorrhizal inoculum contained extramatrical chlamydospores and infected root bits of *Panicum maximum* Jacq. which was infected with *Glomus fasciculatus* (Thaxt.) Gerd. Trappe. The inoculum was placed 2 cm below the soil surface before sowing, to produce mycorrhizal plants. Nitrogen, at the rate of 30 mg per kg of soil, was applied to all the pots as urea. Groundnut plants (6 replicates) were harvested after 10, 20, 30, 45 and 60 days' growth. Fresh root samples were ground well and extracted with ethanol[8]. From the extract thus obtained total and ortho-dihydroxy (O–D) phenols were estimated by the methods of Bray and Thorpe[9] and Johnson and Schaal[10], respectively. Mycorrhizal infection in the root was determined after staining with trypan blue[11]. Roots were fixed in Carnoy's B solution (6:3:1:ethanol: chloroform: acetic acid) for 1 h, dehydrated using an n-butanol series, embedded in 56 °C paraffin and cut into sections of 6 µm thickness. Sections were stained for phenols with 0.05% toluidine blue-O in citrate buffer (pH 4.4) for 5 min[12]. Phenols were also confirmed using ferric chloride and Hoepfner-Vorsatz reagents as outlined by Ling-Lee et al.[13]. Photographs were exposed in a wave length range of 400-600 nm.

Results and discussion. Percentage mycorrhizal infection, total and O–D phenol content of mycorrhizal and non-mycorrhizal groundnut roots are given in the table. There was no difference in the total phenol content of the mycorrhizal and non-mycorrhizal plants at 10 days. No mycorrhizal infection could be detected at this stage. In the mycorrhizal roots total phenol content was initially high (day 10), decreased on day 20 but increased later. A similar trend was found with respect to O–D phenol content, except that it dropped again on day 60. Such changes were absent in non-mycorrhizal roots. Higher concentrations of total phenol in mycorrhizal plants compared to uninoculat-

Phenol content and percentage mycorrhizal infection of the mycorrhizal and non-mycorrhizal groundnut roots

Age of plant days	Total phenol (µg/g fresh weight sample)		O-D phenol (µg/g fresh weight sample)		Mycorrhizal infection (%)	
	M	NM	M	NM	M	NM
10	269.9	247.5	115.1	61.9	0	0
20	218.2	265.2	106.7	71.1	15	0
30	290.7	264.2	130.5	73.1	59	0
45	313.5	281.9	119.6	85.1	72	0
60	417.1	280.9	87.4	77.9	79	0
LSD at p = 0.05	40.0		17.1		ND	

M, mycorrhizal roots; NM, non-mycorrhizal roots; ND, not determined.



Transverse sections of mycorrhizal groundnut root showing deeply stained phenols in arbuscules, and inter- and intracellular hyphae. Toluidine blue. ar, Arbuscule; ia, intracellular hypha; ie, intercellular hypha; c, cortical cells without the fungus. × 827.5.

ed plants from day 30 onwards correlated with the infection of the root system. Histochemical study suggested that the higher amounts of phenol in mycorrhizal plants might be due to deposition of phenols in the fungal structures. Arbuscules, and inter- and intracellular hyphae showed deeply stained phenol deposition compared to cortical cells devoid of the fungus (fig., a, b).

Working with tomato, Dehne and Schönbeck[7] also found that mycorrhizal plants contained higher amounts of phenol compared to uninoculated plants. Initial increase in phenol in mycorrhizal plants is probably due to an incompatible reaction of the host plant to the invading fungus. Similar situations exist when pathogenic fungi invade host plants[14]. Some workers have correlated the presence of phenolics in plants with resistance to pathogens[15]. Mycorrhizal inoculation is known to impart resistance to the host against disease[16]. Another study conducted by us indicated that the concentration of O–D phenol present in mycorrhizal roots inhibited in vitro growth of the root pathogen, *Sclerotium rolfsii*[17]. The reduction in dry weight of mycelium grown in potato dextrose broth for 15 days was 27%. Thus, it seems that the higher amount of phenols might be one of the factors responsible for increased disease resistance found in mycorrhizal plants.

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Development of a new tachykinin antagonist

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Summary. The mouse urinary bladder possesses a tachykinin receptor which responds to kassinin and eledoisin, but not to some other tachykinins. The action of kassinin, but not that of eledoisin, was blocked in a surmountable manner by a new tachykinin antagonist, D-Pro^4, Val^8, D-Trp^{7,9,10} (SP)$^{4-11}$.

Within the last 18 months several substance P (SP) antagonists have been described[2–4]. Although these compounds effectively antagonize SP action on a variety of tissues, several problems are associated with their use; they generally exhibit low pA_2-values, some exert agonistic actions[5,6], an unsurmountable rather than a surmountable inhibition may be observed and they do not antagonize the actions of SP and some other tachykinins on all tissues.

In the present study we have attempted to develop an antagonist for a tachykinin receptor that is highly selective for 2 members of the tachykinin family of peptides: eledoisin (ED) and kassinin (KA). Although there are many structural differences between these 2 peptides and SP (table), we chose to modify position 8 of the antagonist D-Pro^4, D-Trp^{7,9,10} (SP) 4–11, recently described by Mizrahi and coworkers[4]. KA and ED have nonpolar amino acids in position 8; valine and isoleucine, respectively, whereas SP has an aromatic residue, phenylalanine, in this position. The antagonistic properties of these compounds are due to a collection of D-enantomiers at positions 7, 9 and 10, with the amino acid in position 8 being important for binding to the receptor[7].

Material and methods. The actions of the tachykinins, in the presence and the absence of tachykinin antagonists, were tested on strips (2 × 10 mm) of the mouse urinary bladder (Swiss White strain). The tissues were suspended in an 85 µl tissue bath[8] and superfused with oxygenated Krebs solution of the following composition (mM): NaCl 118.7, KCl 4.7,

Structures of several tachykinins and 2 tachykinin antagonists

		1	2	3	4	5	6	7	8	9	10	11
Substance P		Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Physalaemin		Glp-*	Ala-	Asp-	Pro-	Asn-	Lys-	Phe-	Tyr-	Gly-	Leu-	Met-NH_2
Eledoisin		Glp-	Pro-	Ser-	Lys-	Asp-	Ala-	Phe-	Ile-	Gly-	Leu-	Met-NH_2
Kassinin	Asp-	Val-	Pro-	Lys-	Ser-	Asp-	Gln-	Phe-	Val-	Gly-	Leu-	Met-NH_2
Compound I					pro-**	Gln-	Gln-	trp-	Phe-	trp-	trp-	Met-NH_2
Compound II					pro-	Gln-	Gln-	trp-	Val-	trp-	trp-	Met-NH_2

* Glp, pyroglutamic acid; ** D-amino acids are written entirely in small letters.