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Exudates of avocado rootstocks and their possible role in resistance to *Phytophthora cinnamomi*

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ABSTRACT

Root exudates from Edranol roots contained higher concentrations of 14 amino acids than roots of Duke 7, G6 and G755. A combination of these amino acids showed the highest degree of zoospore attraction in vitro*. Edranol roots attracted more zoospores than the more resistant Duke 7, G6 and G755 roots.*

UITTREKSEL

Eksudate van Edranol-wortels het in vergelyking met Duke 7, G6 en G755, hoër konsentrasies van 14 aminosure bevat. In vitro *het 'n kombinasie van die aminosure die meeste soóspore gelok. Edranol-wortels het meer soóspore gelok as die meer weerstandbiedende Duke 7, G6 en G755-wortels.*

INTRODUCTION

Different avocado rootstocks show different levels of tolerance to *P cinnamomi* (Coffey, 1987). Zentmyer (1961) demonstrated a chemotaxtic response of zoospores to a concentration gradient of diffusing substances from roots of avocado seedlings. He also reported that zoospores of *P cinnamomi* were less attracted to roots of cultivars tolerant to *P cinnamomi* than to roots of susceptible cultivars. This phenomenon was confirmed by Aveling (1988) and Botha, Wehner & Kotzé (1989). Different research workers demonstrated chemotaxis of zoospores to compounds in root exudates, eg sugars, amino acids and organic acids (Royle & Hickman, 1964; Rai & Strobel, 1966; Chang-Ho & Hickman, 1970).

In this study, the composition of avocado root exudates was determined and correlated to zoospore attraction by the various rootstocks. Furthermore, constituent parts of root exudates were tested *in vitro* for attraction to *P cinnamomi* using the DM-technique.

MATERIALS AND METHODS

Plant material and inoculum production

Roots, 1-2 mm in diameter, obtained from susceptible 14-month-old *P americana* cv Edranol seedlings (Snyman, Snyman & Kotzé, 1984) and vegetatively propagated

(Frolich & Platt, 1971) seedlings of *P americana* selections of Duke 7 and G6 (moderately tolerant) (Coffey, 1987), as well as *P scheideana*. Selections G755, tolerant according to Coffey (1987), were used in this experiment. *P cinnamomi* (PREM 49103, unknown mating type) isolated from avocado roots collected in the Transvaal (Tzaneen area), was used as test organism. The method of Chen & Zentmyer (1970), with the salt solution of Gisi, Zentmyer & Klure (1980), was used to induce zoospore production. A concentration of 10^5-$10^6 m\ell^{-1}$ zoospores was obtained in this manner.

Zoospore attraction to roots

Zoospore attraction to avocado roots was determined with the dialysis membrane (DM) technique (Botha *et al,* 1989) and with the scanning electron microscope.

Root exudates

Root exudates were collected and concentrated as follows: 90 feeder roots (*ca* 35 mm long, 10 from each of nine seedlings) were collected from each rootstock. Ten roots were placed in each of nine glass vials (7 mℓ) containing 5 mℓ sterile distilled water with their tips suspended in the solution. The vials (with roots) were incubated in the dark at 25°C. After 4 h the roots were removed and their diameters determined in order to standardise results. The nine 5 *mℓ* samples were pooled to form three 15 mℓ samples which were individually lyophilised. For sugar and amino acid determinations, samples were analysed with a high-performance liquid chromatograph (HPLC).

Zoospore attraction to exudates

Chemotaxis of zoospores to various amino acids and sugars, occurring in exudates, were determined *in vitro* with the DM-technique. All the amino acids and sugars were individually made up to a concentration of 1 mM. Amino acid and sugar combinations were also tested. Filterpaper discs (3 mm diameter) were soaked in the different compound solutions and placed on the dialysis membrane instead of the roots. Attraction to the different compounds were determined after 2 h by removing the membranes and staining them with 0,5 per cent cotton blue in lactophenol. Zoospore encystment was examined microscopically.

RESULTS

Edranol roots attracted significantly more zoospores than the other three rootstocks, as tested with the Dm-technique and monitored with the SEM (Table 1). HPLC analysis showed the presence of 14 amino acids in avocado root exudates. More of these amino acids occurred in Edranol root exudates than in exudates of Duke 7, G6 and G755. Total amino acid concentration in exudates of each rootstock correlated with zoospore attraction to roots of these rootstocks (Table 2). The 14 amino acids were tested *in vitro* individually and in combination in an effort to identify the amino acid (or amino acids) that caused the chemotaxtic response. Individual amino acids (with the exception of

glutamic acid) had little or no chemotaxtic effect on zoospores while the amino acid combination (1 mM) attracted a high amount of zoospores (Table 3).

TABLE 1 Zoospores encystment of *P cinnamomi* on root tips of four avocado rootstocks determined by means of the dialysis membrane technique and the SEM

Rootstock	Number of cysts per root tip*	
	Dialysis membrane technique	SEM
Edranol	82a	123a
Duke 7	17b	62b
G6	6b	57b
G755	2b	26b

*In columns values not followed by the same letter, differ significantly according to Duncan's multiple range test (P=0,05).

TABLE 2 Presence of various amino acids and sugars in the root exudates of four avocado rootstocks

Compound	Concentration (mM/sample in exudate) from rootstock			
	Edranol	Duke 7	G6	G755
Alanine	13,1	6,6	3,3	1,9
Serine	6,5	5,3	2,6	1,0
Glycine	5,6	4,3	1,7	0,8
Valine	4,1	3,3	1,7	0,4
Proline	3,9	1,7	0,9	0,1
Y-amino butyric acid	3,7	A	0,1	0,5
Leusine	2,9	2,0	1,7	0,4
Isoleusine	2,8	1,6	1,2	1,3
Tyrosine	1,4	0,9	0,6	0,1
Arginine	1,2	0,4	0,2	A
B-amino-iso-butyric acid	1,0	0,8	0,4	0,5
Aspartic acid	0,5	A	0,2	0,1
Gluatmic acid	0,9	0,3	A	A
Phenyl alanine	1,4	0,1	0,1	0,2
Glucose	NR	NR	NR	NR
Sucrose	NR	NR	NR	NR
Fructose	NR	NR	NR	NR
Total amino acids	49,0	27,3	14,7	7,3
Total sugars	NR	NR	NR	NR

A = not detectable NR = no meaningful results

TABLE 3 Attraction of *P cinnamomi* zoospores to compounds occurring in avocado root exudates measured with the dialysis membrane technique

Compound	Number of cysts per membrane attracted to 1 mM concentrations of the various compounds
Alanine	1,7
Serine	0,7
Glycine	0,3
Valine	0,3
Proline	1,7
Y-amino butyric acid	3,3
Leusine	2,0
Isoleusine	2,0
Tyrosine	0
Arginine	0,7
B-amino-iso-butyrid acid	2,0
Aspartic acid	3,0
Glutamic acid	6,0
Total amino acids	15,0
Sucrose	2,0
Glucose	1,3
Fructose	1,0
Total sugars	0,7
Total amino acids + sugars	11,0
Roots	29,0
Control	0

DISCUSSION

Zentmyer (1961) reported that zoospores of *P cinnamomi* were less attracted to roots of avocado cultivars resistant to *P cinnamomi* than to roots of susceptible cultivars. The same phenomenon occurred in this study with Duke 7, G6 and G755, attracting significantly less zoospores than the susceptible Edranol rootstock. The reported greater attraction of zoospores to susceptible avocado cultivars (Zentmyer, 1961), was corroborated in the present study.

Analysis of root exudales showed that Edranol root exudates contained higher amino acid concentrations than the other three tolerant rootstocks. Although Zentmyer (1961) theorised this, it was proven for the grapevine/*P cinnamomi* interaction (Marais & Hatting, 1985).

Zoospores of *P cinnamomi* were more attracted to a solution containing the 14 amino acids detected in avocado root exudates than individually to these amino acids. Sugars showed little or no attraction. Glutamic acid did however; attract more zoospores than the other single amino acids. This correlated with results obtained by Khew & Zentmyer (1973), who found that arginine, aspartic acid and glutamic acid were more attractive to zoospores than other amino acids.

The exudation of different amino acids in different concentrations could be an important factor in determining tolerance or susceptibility of different avocado rootstocks to *P cinnamomi.*

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