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PRODUCTION OF FLAVOUR PRECURSORS [*S*-ALK(EN)YL-L-CYSTEINE SULPHOXIDES] IN PHOTOMIXOTROPHIC CALLUS OF GARLIC

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Abstract—Intact garlic plants contain three flavour precursors, *S*-allylcysteine sulphoxide, *S*-propylcysteine sulphoxide and *S*-methylcysteine sulphoxide. Undifferentiated white callus contains only *S*-methylcysteine sulphoxide. Full flavour precursor expression, comparable to intact plants can be induced in green undifferentiated callus, in redifferentiated green roots and in redifferentiated green shoots. Redifferentiated white roots contain *S*-allyl-, *S*-propyl- and *S*-methylcysteine sulphoxides, but at very low levels. It is suggested that *S*-methylcysteine sulphoxide is constitutively expressed in garlic callus cells, but that the expression of the biosynthetic pathway to *S*-allyl- and *S*-propylcysteine sulphoxides requires the presence of differentiated plastids or, optimally, chloroplasts.

INTRODUCTION

Allium species characteristically contain a high concentration of non-protein sulphur amino acids (between 1 and 5% dry wt). One class of these secondary metabolites, the *S*-alk(en)yl-L-cysteine sulphoxides gives rise to the characteristic aroma and flavour of *Allium* species. In the intact cell the sulphoxides (flavour precursors) are compartmentalized in the cytoplasm and the hydrolytic enzyme alliinase in the vacuole [1]. Disruption of cellular compartmentalization results in release of alliinase, and the subsequent hydrolysis of the sulphoxides to volatile sulphides. Onions (*Allium cepa*) contain *S*-propenyl, *S*-propyl- and *S*-methylcysteine sulphoxides, whereas garlic (*Allium sativum*) contains *S*-allyl; *S*-propyl- and *S*-methylcysteine sulphoxides. *S*-Allylcysteine sulphoxide gives rise to the characteristic garlic taste. *S*-Propyl- and *S*-methylcysteine sulphoxides provide additional flavour notes [2].

Although flavour is detectable in all parts of intact onion and garlic plants [2], it is not detectable in either garlic callus cultures [3] or onion callus cultures [4, 5]. Callus of both species produces the hydrolytic enzyme alliinase [3, 4] but only *S*-methylcysteine sulphoxide, a minor flavour note [3–5]. Full flavour was lacking because of the absence of *S*-allyl and *S*-propylcysteine sulphoxide in garlic callus and *S*-propenyl- and *S*-propylcysteine sulphoxide in onion callus. Redifferentiation into shoots and roots restored full flavour precursor expression [6].

We now report the expression of *S*-allyl- and *S*-propylcysteine sulphoxides, and thus flavour, in photomixotrophic callus cultures of garlic.

RESULTS AND DISCUSSION

Intact green leaves from garlic plants contained the flavour precursors *S*-allyl, *S*-propyl- and *S*-methylcysteine sulphoxides and had a characteristic garlic taste. Undifferentiated light-grown, white callus had almost no taste and contained only *S*-methylcysteine sulphoxide at 0.08 mg/g fresh weight (Table 1). However, callus in which chlorophyll had developed (photomixotrophic) had a characteristic garlic taste, and upon analysis showed the presence of *S*-allyl/*S*-propylcysteine sulphoxides as well as *S*-methylcysteine sulphoxide at levels comparable to intact plants. Green roots and green shoots which had differentiated from callus also contained the full complement of flavour precursors. White roots, even when grown in the light, contained all three flavour precursors but only at very low levels, and tasted minimally of garlic.

Undifferentiated non-green cells of *Allium* species, grown in tissue culture, produce minimal flavour when they are cut or eaten. The enzyme alliinase has been shown to occur in onion callus and in garlic callus although at slightly lower levels in the latter compared to intact garlic plants [3–5]. An analysis for flavour precursors showed that only *S*-methylcysteine sulphoxide was present and at lower concentrations than in the intact plant [5]. Thus the lack of flavour in callus was attributed to low levels of flavour precursors. However, when callus redifferentiated into roots and shoots, flavour precursor synthesis occurred at an increased level and flavour was detectable [3, 4, 6].

Turnbull *et al.* [6] studied the ultrastructure of onion callus and suggested they lacked some structural feature associated with differentiation that allowed synthesis of flavour compounds. The results obtained with photomixotrophic callus presented above indicated that plastid differentiation, and optimally chloroplast formation is necessary for flavour precursor expression.

Chloroplast formation, as indicated by photomixotrophic and photoautotrophic cultures, has been found to be necessary for the expression of quinolizidine alkaloids in *Lupinus polyphyllus* [7], of lipoquinones in *Morinda lucida* [8] of limonene in *Apium graveolens* [9], and of solasodine in *Solanum laciniatum* [10]. In each of these examples the compounds mentioned were synthesized in green leaves in the intact plants. In intact onion plants, green leaves function as the major site of synthesis of

Table 1. Production of flavour precursors by garlic cultures 12 days after transfer to light conditions

Tissue analysed	weight (g)	Flavour precursors (mg/g fr. wt) methyl CS	allyl/propyl CS
Callus, white	1.93	0.08	0.00
Callus, green	0.48	0.19	0.37
White roots	2.54	0.03	0.04
Green roots	1.29	0.15	0.39
Green shoots	0.61	0.18	0.30
Intact leaves (control)	—	0.1–0.6	0.5–2.6

$n=2$.
CS = Cysteine sulphoxide.

flavour precursors [11]. However, bulbs and roots of both onion and garlic have the capacity to synthesize flavour precursors [12–14].

These results with intact plants parallel the situation in culture whereby differentiation of white roots produced very low levels of all flavour precursors. However, in both differentiated and undifferentiated cells chloroplasts were required for optimal levels of all flavour precursors.

Chloroplasts/plastids do not appear to be required for the synthesis of *S*-methylcysteine sulphoxide. This suggests a basic difference in the regulation of this flavour precursor as compared with the others. It appears that *S*-methylcysteine sulphoxide is constitutively expressed in all of the *Allium* cells which have been studied.

The exact role of plastids/chloroplasts in the formation of *S*-allyl and *S*-propylcysteine sulphoxides is unclear. They may be the site of all or part of the biosynthetic pathway to these flavour precursors. Alternatively, plastid differentiation may provide the right developmental signal for the induction of the biosynthetic enzymes in the cytoplasm.

EXPERIMENTAL

Plant tissue culture. Garlic (cv Y-strain) cloves were surface sterilized by rinsing with surfactant (Teepol) in sterile H_2O, dipping in 95% EtOH (60 sec) washing in 1% NaClO (15 min), and rinsing × 3 in sterile distilled H_2O. Cloves were cut lengthways and widthways and the tips and middle sections were plated on the basal medium BDS [15] containing 6.21 μM 4-amino-3,5,6-trichloropicolinic acid (Picloram) and 8.88 μM 6-benzyl-aminopurine (BA). Callus was induced after 4–6 weeks on this medium in the dark at 25°. After the callus had become well established it was subcultured *ca* every 8 weeks onto the same medium. At the time of extraction, the callus had been cultured for 13 months in total. During this time some of the calli had redifferentiated to form shoots or roots. Twelve days prior to analyses different tissue types were selected, and transferred to light (75 μmol/m^2/sec; 16 hr photoperiod) at 24°. Some of the roots and calli, and all of the shoots, produced chlorophyll within seven days. Those roots and calli which did not produce chlorophyll remained white or pale yellow in colour, comparable to dark-brown roots and calli. Samples were selected to represent combinations of undifferentiation/redifferentiation and greened/white. (1) White, friable callus, (2) green, dense callus, (3) redifferentiated white roots on callus, (4) redifferentiated green roots on callus, (5) redifferentiated green shoots on callus. Redifferentiated roots and shoots were cut from the calli prior to analyses. All samples were weighed and subsequently analysed for flavour precursor content.

Analysis of flavour precursors. Tissue was extracted twice in MeOH–$CHCl_3$–H_2O (12:5:3) and once in 80% EtOH. The EtOH–H_2O phase was separated on Dowex-1 ion-exchange chromatography. *S*-Alk(en)yl-L-cysteine sulphoxides were eluted with 0.1 M HOAc and further purified on cellulose plates by electrophoresis. They were separated by TLC on Merck silica gel plates in solvent I (MeCOEt–pyridine–H_2O–HOAc, 80:15:15:2) and then solvent II (*n*-PrOH–H_2O–PrCOAc –HOAc-pyridine, 120:60:20:4:1) in the same direction. Visualization of the sulphoxides was with ninhydrin and quantification was by scanning densitometry. Recoveries were computed by the addition of known amounts of *S*-butylcysteine sulphoxide to each extract. Analytical details are described in ref. [16] and Shaw, M. L., Lancaster, J. E. and Lane, G. A. (in preparation).

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