Chemical Composition of Chickpea, *Cicer arietinum*, Exudate

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The exudate dry matter of the *Cicer arietinum* genotype ICC 506 is composed of three main organic acids, malate (61.2%), oxalate (28.6%), and glucose-6-phosphate (5.5%). Minor components are citrate (2.5%), succinate (1.2%), malonate (0.6%), oxalacetate (0.2%), and fumarate (0.2%).

Introduction

The chickpea, *Cicer arietinum* L., secretes a highly acidic exudate which has a pH near to 1.0. It is released through trichomes which are located on the plant's whole green surface, including the pods. The exudate seems to be involved in chickpea resistance against one of its main insect pests, *Helicoverpa (Heliothis) armigera*, the gram pod borer [1]. Malate, the main organic acid in the chickpea exudate, cannot be responsible for such a low pH value, however. We therefore conducted a complete analysis of an exudate which had been collected from a chickpea field under the semi-arid conditions of India.

Materials and Methods

The exudate was collected at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at Hyderabad, India. From the genotype ICC 506 which is highly resistant against attack by *H. armigera*, the exudate was wiped off with cotton plugs which then were squeezed out into glass vials. These were subsequently sealed with paraffin and immediately stored in the refrigerator. By that procedure the slightly yellow exudate had the same concentration as it was present on the plant surface.

The exudate was analyzed by HPLC under the following conditions. Instruments from Perkin-Elmer, Series 3 B-Liquid Chromatograph and from Hewlett-Packard, Diode-Array (System 1040 A), Computer and Terminal No. 300, HP 79995 A and Printer No. 7475 A. The HPLC column used was a Polyspher^R-OA-KC column (Merck), 300 mm length, 7.8 mm i.d. with the solvent 0.01 N H_2SO_4 [2–4] and a flow rate of 0.4 ml/min.

For an additional qualitative identification of the exudate components, HPTLC silica plates (Merck) were used with the following solvent: butanol(1)–acetone–acetic acid–ammonia(33%)–water (35:15:15:2.5:27.5) [5]. For detection the following reagents were used: Schweppes reagent [5], Hanes' reagent [5], Fehling's solution [6], molybdophosphate (Merck No. 531) and ninhydrin (Merck No. 6758) reagents.

Enzyme tests were used for quantification of glucose (Sigma-Diagnostics, Glucose(HK) 10-No. 16–10) and of malate (Boehringer, No. 139068).

Results and Discussion

Eight different organic acids were identified by use of HPLC. Their retention time and concentration in the ICC 605 exudate are collated in Table I. The column which was used for this analysis, perfectly separates all the acids by base line. No additional UV absorption was visible over the whole chromatogram.

Besides these eight organic acids, two more compounds, glucose and phosphate, were quantified by the respective assays. The result out of three replicates is shown in Table II.

Table I. Retention time and amount of organic acids contained in the exudate of *C. arietinum* ICC 506. Separation on a Polyspher-OA-KC column. For more details see Materials and Methods.

Compound	Retention time (min)	Amount (mg/ml)	Amount (mol/ml)	%
Glucose-6-P	7.67	2.25	8.33	5.5
Oxalate	8.90	3.80	43.00	28.6
Citrate	12.70	0.60	3.75	2.5
Oxalacetate	14.72	0.042	0.32	0.2
Malate	16.80	119.60	92.43	61.2
Malonate	17.42	0.09	0.96	0.6
Succinate	21.47	0.21	1.80	1.2
Fumarate	28.98	0.04	0.31	0.2

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Table II. Concentrations of glucose and phosphate in the ICC 506 exudate.

Compound	Concentration (mol/ml)
D-glucose	8.38
Phosphate	9.85

Both these compounds are present in the exudate in a proportion of about one by one, indicating that they may be derived from glucose-6-phosphate which is present in the exudate in about the same concentration as its two components.

All the acids were first identified by comparison with the original pure materials by use of TLC, enzyme test and, respectively, by phosphate tests. The HPLC technique as applied in this exudate analysis is highly sensitive and selective. Consequently, it is ideal for routine analyses of different chickpea genotypes. Whereas former studies [7, 8] titrated the exudate and from this value calculated total acid as malate, we now demonstrate the presence of at least three main acids in the exudate (Table I).

Whereas malate and oxalate represent the two main dicarboxylic acids present in the exudate, glucose-6-phosphate is responsible for its extremely low pH. This metabolite does not originate from such trichomes or other cells which could have been damaged during exudate collection. To prove this, chickpea leaves were washed with water, and glucose-6-phosphate was also found in this case. All the other acids present in the exudate make up less than five percent of the total (Table I). It will now be interesting to analyze chickpea genotypes which differ in their susceptibility to *H. armigera* attack for their differences in the concentration of the three main exudate factors. Our former studies have already indicated that there seems to exist a correlation between malate concentration, as the main exudate component, and pest insect resistance [1].

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