

RECUPERAÇÃO DE ÁREAS DEGRADADAS E ABANDONADAS, ATRAVÉS DE SISTEMAS DE POLICULTIVO

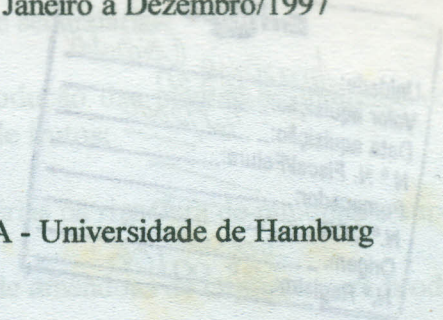
PERÍODO: Janeiro a Dezembro/1997

Embrapa/CPAA - Universidade de Hamburg

Editores:

Luadir Gasparotto & Götz Schroth

634.99
25552
1997



99
r
998.00106

Recuperação de áreas
1998 RT-1998.00106



3817-1

Manaus-AM
Maio/1998

PROTEIN PATTERNS OF RIPE SEEDS OF *Theobroma cacao*, *Theobroma grandiflorum*, AND *Theobroma bicolor*.

Christoph Reisdorff

Aparecida G. Claret de Souza

Reinhard Lieberei

Böle Biehl

1. INTRODUCTION

Theobroma cacao, the cocoa tree, became one of the main tropical crop plants due to the unique and delicious flavour of the product obtained from the seeds: raw cocoa, the base for the production of chocolate and related products which are consumed world wide. In Brazil cocoa trees widely suffer from the causal agent of witches broom, inflicting severe losses on the Brazilian cocoa production.

T. grandiflorum, the cupuaçu-tree, is considered an excellent crop for local adapted land use systems on former terra firme rain forest areas in the Amazon region. It has been shown that the cultivation in mixed cropping systems favours the development and productivity of cupuaçu-trees. The fruit pulp fetches relatively high market prices whilst the seeds, which amount up to 20% of the fruit's fresh weight, are not yet commercially used. Attempts to commercialise a type of chocolate-wares made from cupuaçu-seeds failed so far, probably due to the unsatisfactory and hardly reproducible quality of the product.

T. bicolor, which is reported to be a very robust tree, can be found nearly throughout the humid tropics of central and south America. In some regions the seeds are used for the production of chocolate for one's own consumption and for local markets.

The objective of the studies is to evaluate whether and under what conditions the seeds of *T. grandiflorum* and *T. bicolor* have the potential to develop a good chocolate-like aroma, revealing the option of producing a storable and valuable ware (in addition to the pulp in the case of cupuaçu).

It has been shown by BIEHL and co-workers that the precursors of the cocoa-aroma are formed from seed storage globulins by enzymatic digestion during fermentation. Thus, as a first approach we studied the seed proteins of the three species by denaturing poly-acryl-amide gel electrophoresis, paying special attention to the globulins.

2. MATERIAL AND METHODS

Fruits of *T. grandiflorum* and *T. bicolor* have been taken from the living collections and the SHIFT-experimental site at the Embrapa/CPAA, Manaus, Brazil.

The cocoa fruits (variety "Mexican Criollo") have been provided by Mrs. Frances Bekele from the Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad and Tobago.

After careful removal of the seed coat the embryos have been shock-frozen by liquid nitrogen. Subsequently the embryos have been freeze dried until the weight of the samples remained constant.

The seed fat of the freeze dried and ground material has been exhaustively extracted by petrol ether (Bp. 40-60°C) in a Soxhlett apparatus. The phenolic compounds have been removed by means of stepwise extraction with aqueous acetone (80% and 70%) containing 5 mmol/l sodium ascorbate or 1 % (v/v) thioglycolic acid. Final extraction by 100 % acetone and subsequent removal of the solvent by low pressure led to the completely water free powder (acetone dry powder, acdp).

In order to obtain the albumin fraction of the seed proteins the acdp has been extracted by low salt buffer (0.01 mol/l NaCl, 0.05 mol/l TRIS/HCl pH 7.5, 2 mmol/l Na₂-EDTA, 7 mmol/l 2-mercaptoethanol). The precipitate was extracted by a high salt buffer (0.5 mol/l NaCl, 0.2 mol/l TRIS/HCl pH 8.0) in order to obtain the globulin fraction.

For the analysis by sodium-dodecyl-sulfate gel electrophoresis of the albumin's and globulins the fractions have been diluted by SDS sample buffer (1:2) containing 8 mol/l urea and 2 % SDS. For the entire seed protein pattern the acdp has been extracted directly by SDS sample buffer. The electrophoresis has been conducted using BIO-RAD 10-20% poly-acrylamid gradient ready gels. The protein bands have been stained by Coomassie Brilliant Blue or by silver staining methods.

3. RESULTS

The patterns of seed proteins of all three *Theobroma* species are marked by two predominant globulins and at least one predominant albumin. The molecular weights of the two classes of globulins are quite similar for all three species (49 ± 3 kDa and 33 ± 1 kDa). Considering the quantity of globulins the seeds of *T. bicolor* are prominent regarding their very high content of these storage proteins, while the seeds of *T. grandiflorum* are the only ones containing more albumins than globulins. However, in all three species the relative quantity of the globulins at the range between 32 and 34 kDa vary around 15% of total protein content (Table. 1, Figure. 1).

The results give rise to the suggestion that *T. grandiflorum* and *T. bicolor* meet at least one requirement for the generation of a chocolate like aroma: the seeds contain globulins similar to those of cocoa regarding the molecular weights and the relative quantities.

The degree of similarities will be further studied by investigating molecular details of the globulins through immune-serological and enzymatic techniques. Additionally we are studying the proteases which are responsible for the post-mortem degradation of the globulins leading to the flavour precursors of cocoa-aroma.

TABLE 1. Specifications of seed proteins (predominant globulins and albumins) of three *Theobroma* species.

Protein	Species	Molecular Weight (kDa)	Quantity (% of total protein)
globulin 1	<i>T. grandiflorum</i>	52 Da	2.4
	<i>T. cacao</i>	51 kDa	9.5
	<i>T. bicolor</i>	46 kDa	32.9
globulin 2	<i>T. grandiflorum</i>	32 kDa	13.4
	<i>T. cacao</i>	34 kDa	12.5
	<i>T. bicolor</i>	33 kDa	16.9
albumin 1	<i>T. grandiflorum</i>	23 kDa	30.4
	<i>T. cacao</i>	22 kDa	19.5
	<i>T. bicolor</i>	20 kDa	5
albumin 2	<i>T. grandiflorum</i>	12 kDa	10.3
	<i>T. cacao</i>	-	-
	<i>T. bicolor</i>	16 kDa	14.8
albumin 3	<i>T. grandiflorum</i>	-	-
	<i>T. cacao</i>	-	-
	<i>T. bicolor</i>	15 kDa	4.5

The relative quantity of proteins has been determined by analyzing densitometrically the SDS-PAGE pattern of seed proteins using a Sharp JX 325 scanner and the Imagemaster® software of Pharmacia.

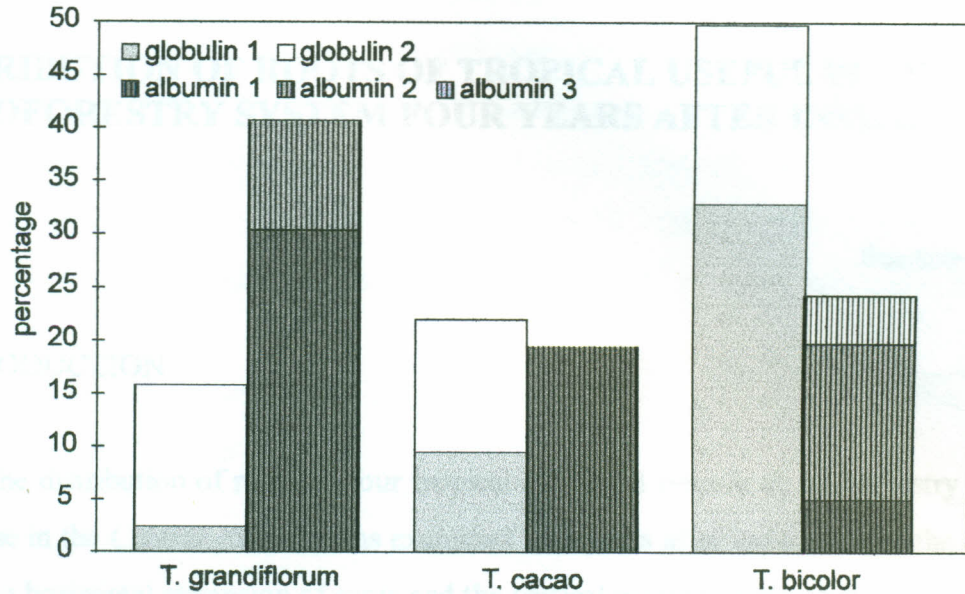


Figure. 1: relative quantity of the predominant globulins and albumins in the cotyledons of three *Theobroma* species (percent of total protein; cf. tab. 1).