Rekultivierung degradierter, brachliegender Monokulturflächen in ausgewogene Mischkulturflächen unter besonderer Berücksichtigung bodenbiologischer Faktoren

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HISTOLOGICAL AND CYTOLOGICAL STUDIES ON SEEDS OF *THEOBROMA* SPECIES

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1. INTRODUCTION

All species of of the genus *Theobroma* L. produce berry fruits with hard shells and large seeds which are embedded in a pulp. Among the 22 species only *Theobroma cacao* L., the cocoa tree, plays a significant economical role. Cocoa seeds are used for the industrial production of cocoa and chocolate as well as for cocoa butter, which is an ingredient of many cosmetical and pharmaceutical products and food.

Besides *T. cacao*, the seeds of at least 12 other *Theobroma* species are qualified as raw material for an industrial manufacture of cocoa-like products. This can be derived from their use by ethnical groups in confined areas of the amazonas basin.

The use of seeds of the Cupuaçu tree (*Theobroma grandiflorum* (WILLD. EX SPRENG.) SCHUM.) for an industrial production of „cupulate" (chocolate from Cupuaçu seeds) would have positive socioeconomic effects for the whole Amazon Region as there already exists a widespread cultivation of Cupuaçu in this area. Actually, mainly the pulp of the Cupuaçu fruits is used as a popular ingredient of juices and sweets while the seeds in general are discarded. The establishment of a „cupulate" industry would increase the market value of Cupuaçu fruits significantly.

Cocoa-specific aroma precursors are generated during fermentation of *Theobroma* seeds by an acid-induced proteolysis of seed proteins by endogenous proteases. The seed acidification during the fermentation process is caused by microbial production of acetic acid as a consequence of the pulp components.

The fermentation process is significantly influenced by the histological and cytological structure of the seeds. Thus, in order to develop adequate fermentation processes, the seed structure of other *Theobroma* species must be compared with the structure of cocoa (*T. cacao* L.) seeds.

2. MATERIAL AND METHODS

Seeds of the species *T. cacao* L., *T. grandiflorum* (WILLD. EX SPRENG.) SCHUM. and *T. bicolor* H. B. K. were examined.

Ultra-thin and semi-thin sections were taken from the tissue of the seed cotyledones and were analized by light microscope and transmission electron microscope. Semi-thin sections from the seed shells were examined by light microscope.

3. RESULTS

3.1 THE STRUCTURE OF THE SEED SHELLS

The seed shells of all three examined *Theobroma* species consist of two integuments. The outer integument is much thicker than the inner integument. Inside its parenchyme, it contains vascular bundles and, beneath the outer epidermis, large spaces filled with slime. The parenchyme of the inner integument consists of only a few cell layers. Its outer epidermis is composed of thick-walled, lignified cells.

Significant differences between the three examined *Theobroma* species were found in the thickness of the seed shells (Fig. 1). While the testa of *T. cacao* is only about 385 μm thick, the one of *T. grandiflorum* is 520 μm thick and the seed shell of *T. bicolor* is at least 1670 μm thick.

The thickness of the outer epidermis of the inner integument varies significantly between the three *Theobroma* species (Fig. 1). In the testa of *T. cacao* the epidermal cells form a sclereide layer that is about 8,6 μm thick. The homologue palisade layers of the two other examined species are 28,6 μm (*T. grandiflorum*) and 127,3 μm (*T. bicolor*) thick.

3.2 THE STRUCTURE OF THE COTYLEDONES

Almost the whole volume of *Theobroma* seeds is filled by the two large cotyledones of the embryo which are different in size and strongly folded.

The tissue of the cotyledones mainly consists of storage cells which are ellipsoide, with a length of 15-35 μm. Besides 10 % of cytoplasma, the storage cells contain about 60% fat, 20% proteins and 5-10% starch in seperated compartiments.

Besides the storage cells, polyphenol cells can be found in the tissues of *T. cacao* and *T. grandiflorum*, in a portion of 8-11% in all of the cotyledonar cells. The idioblasts contain a

large vacuole in which phenolic substances are stored. Usually the phenol cells are a bit larger than the surrounding storage cells.

While the distibution of the polyphenol cells of *T. grandiflorum* is equal in the whole tissue, the idioblasts of *T. cacao* mainly appear close to the upper epidermis of the cotyledones where they often form small groups and rows.

The cotyledonar tissue of *T. bicolor* is free of polyphenol cells.

4. DISCUSSION

Significant differences between the three examined *Theobroma* species relative to the fermentation process can be derived from the structural differences shown in the results.

As the seed shells of *T. grandiflorum* and especially of *T. bicolor* are thicker than the one of *T. cacao*, it can be assumed that they have a lower permeability for acids and other substances that pass the seed shells during fermentation. The different sizes of the sclereide layers respectively the palisade layers in the seed shells of the three species confirm this assumption. This will probably retard the seed tissue acidification of *T. grandiflorum* and *T. bicolor* during fermentation, which is essential for the enzymatic formation of the aroma precursors.

As the polyphenol vacuoles in the cotyledonar tissue are destroyed during fermentation, their contents significantly tan portions of the surrounding proteins. Though the function of the products resulting from the tanning process as flavour components is disputed there is no doubt that the tanning itself effects the fermentation process. Thus it can be concluded that the differences between the three examined *Theobroma* species in presence and distribution of polyphenol cells in the cotyledonar tissue result in differences in their fermentation processes.

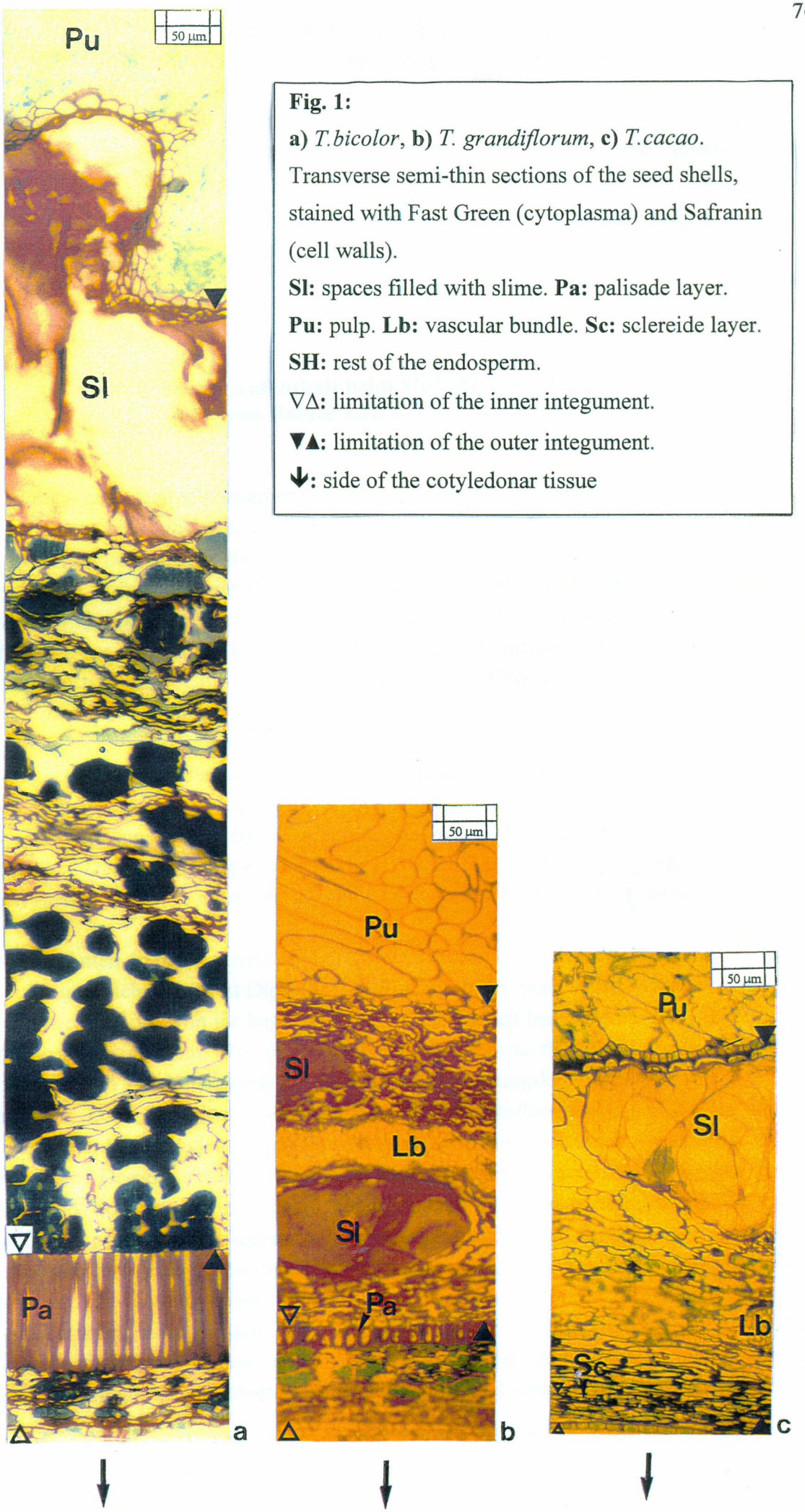

Fig. 1:

a) *T.bicolor*, **b)** *T. grandiflorum*, **c)** *T.cacao*.
Transverse semi-thin sections of the seed shells, stained with Fast Green (cytoplasma) and Safranin (cell walls).

Sl: spaces filled with slime. **Pa:** palisade layer.
Pu: pulp. **Lb:** vascular bundle. **Sc:** sclereide layer.
SH: rest of the endosperm.
▽△: limitation of the inner integument.
▼▲: limitation of the outer integument.
🡻: side of the cotyledonar tissue