

The sustainable production of cyanophycin as multipurpose polymer platform in tobacco combines plant and industrial biotechnology to increase the value of commercially grown tobacco with products that can sustainably substitute fossil raw materials. We aim to establish an economically feasible production system for the biopolymer cyanophycin (CGP) as a byproduct without relevant additional costs, which can be adopted by farmers and biotech companies in Argentina.

CGP is a biopolymer of  $\beta$ -Asp-Arg dipeptides, which is produced naturally by cyanobacteria and can be seen, potentially, as both a valuable N-rich ingredient for the feed industry and as a novel biopolymer for the chemical and material industry. The heterologous production of CGP has already been established in bacteria, yeast and tobacco plants with yields up to 40%, 21% and 9.4% dry weight, respectively. None of the expression strategies were translated into an industrial production system or brought to market application.

This project aims at achieving its goals with a consortium of five expert research centers and industries in the area of tobacco and CGP, namely Bioceres from Argentina, University of Rostock and the Leuphana University from Germany, Wageningen University and Research Centre from the Netherlands and Idroedil from Italy. The project tasks have been organized in three Workpackages (WP's) focusing on 1. Optimization of CGP production by existing CGP-producing tobacco varieties, efficient CGP isolation from tobacco and development of CGP application, 2. Introduction of CGP-production in biodiesel/oil-producing tobacco (Solaris) and implementation of these novel CGP-tobacco cultivars in tobacco-growing sites in Argentina and 3. Social and Economic impacts of the novel CGP-producing tobacco cultivars.

In WP 1, CGP-production by modified Virgin and Burley tobacco will be optimized in greenhouses and on the field. Seeds will be targeted as source for the produced CGP. For optimal extraction of CGP, a biorefinery concept will be developed based on [the](#) Grassa/WFBR technology developed for extraction of proteins and other nutrients. The isolated CGP will be used either as ingredient/N-source for the feed industry as was demonstrated before for CGP-producing potato, ~~or will be used as such as a~~ biopolymer ~~as such, -or as a~~ basis for novel biopolymers after chemical conversion to diaminobutane, ~~and/or~~ for production of polyaspartate after mild hydrolysis (to aspartate and arginine). These novel biopolymers will be tested alone, in blends/mixtures or in combination with other biopolymers such as starch, cellulose and lignin, some of which are also components of the tobacco plant.

In WP 2, CGP production will be introduced in the Solaris variety of tobacco. This variety has been developed for its ability to grow under marginal conditions and for its high oil-content. This tobacco oil is seen as a potential, local, source for bio-jet fuel. By combining the production of biodiesel, protein, fiber and CGP, the economics for this process should become more favorable.

Finally, in WP 3, the economics and social impact of CGP-production by the different tobacco varieties will be assessed. A socio-economic, partial equilibrium, model will be developed to predict the effect of CGP-production, farming, processing, retailing, etc. in Argentina and in the rest of the world. In addition, a Technical Economic Evaluation and Life Cycle Analysis will be conducted for the process of CGP-production by tobacco. For this, the production costs for CGP

by tobacco will also be compared with other existing CGP-producers such as the Cyanobacteria, the modified yeast (*Saccharomyces cerevisiae*) and the modified potato.

With this approach, the consortium expects to develop strong alternatives for the tobacco industry, in general, and especially to geographical areas such as (Northern) Argentina that rely strongly on the growth of this crop.

The processes and outcomes of the project will be aligned with socio-economic issues of the countries involved, thereby providing knowledge for implementation.