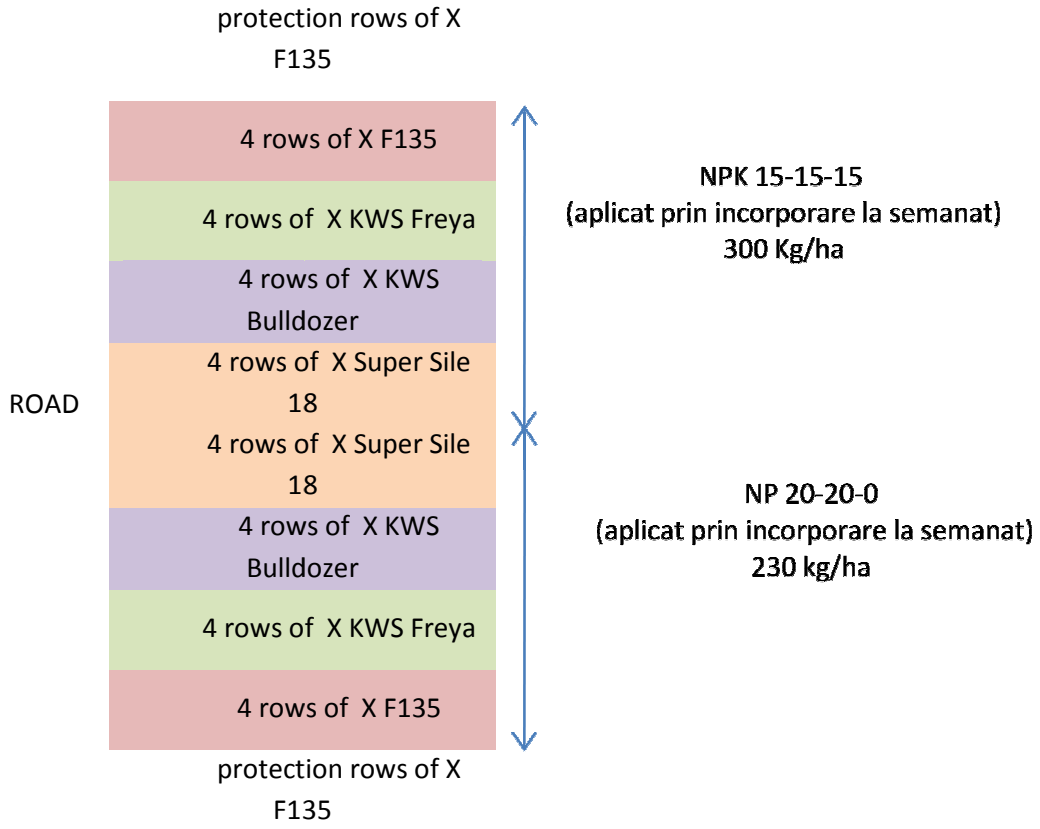
Intermediary Scientific Report

Synthesis

Phase January to December 2014

Team members were continued researches regarding production capacity of sweet sorghum biomass. For this purpose, experimental fields were established containing 4 sorghum hybrid in two versions fertilization and 4 repetitions, in the specific climatic and technological conditions of the lower plains in Timis. Crops were seeded in 04/29/2014, applying a seeding density of 166,000 seeds / ha.



Location Scheme of experimental crops in Timiş County.

In addition, we established experimental fields consisting of 3 hybrids of sorghum in specific climatic and technological conditions of Transylvanian plateau area, on polluted soils in Copşa-Mica area, Sibiu County. The Copşa-Mica experimental groups were performed with hybrids grown in Timiș county in 2013 (var. Jumbo, Sugargraze, Sugargraze II). Analyzes were performed on soil quality from plant emergence to flowering and harvest for the following parameters: pH, total nitrogen, humus phosphorus, potassium sulphate, sodium, calcium and magnesium, for monitoring the dynamics of the absorption of nutritional elements involved in the production of the biomass. In terms of biomass, were obtained values ranging from 16.5 t /

ha dry matter in Freya hybrid (KWS) and 42.8 t / ha dry matter in Bulldozer hybrid (KWS) in the fields with 20-20-0 NPK fertilization.

With regard to the extraction of juice from sorghum, were obtained yields ranging from 0.02 to 0.07 g juice / g in the case of sorghum var. Bulldozer and Freya respectively and from 0.23 to 0.27 g juice / g in the case of sorghum var. Jumbo and Sugargraze respectively. The concentrations of the reducing sugar in the juice extracted vary between 22.13 to 31.80 g / l in the case of var. Super Sile, respectively Sugargraze and up to 70.07 to 84.52 g / l in the case of var. F135 and Sugargraze II respectively.

As regards the preservation of carbohydrates in harvested biomass of sorghum, several experimental variants were made consisting of sorghum silos preserved by administration of different silage additives (selected cultures of lactic acid bacteria, an organic acid and an alcohol in different concentrations). Biomass harvested in autumn 2013 was initially frozen until opening 2014 phase of the project in and in spring 2014 mini-silos were ensiled in laboratory scale. 18 variants of silages were made containing shredded biomass to max. 2 cm. After the addition of preserving additives, the bags were sealed under vacuum, placed in a climatic chamber and stored at 22^{o}C for 6 months. To determine how were preserved sugars in ensiled biomass, juice was extracted from silos at different storage times and determined the concentration of total sugars, reducing sugars, sucrose and glucose. Also, microbiological analyzes were performed to determine the presence and number of microorganisms throughout the shelf-life of biomass with different additives. There were obtained remarkable and surprising results. Under certain conditions of conservation, one of additives not only induced preservation of carbohydrates in biomass silage, but even contributed to extract sugars from biomass into liquid and obtain a higher amount of sugar per quantity of biomass than for biomass pressed before ensiling. Currently, investigations were resumed by diversifying additive concentrations, the biomass harvested in September is currently ensiled and will be sampled up to 6 months to determine conservation capacity (and extraction) of sugars in ensiled biomass.

Regarding the second generation ethanol obtained from lignocellulosic biomass

Fresh harvested biomass was grinded with laboratory mill through 20 mm mesh and juice was extracted by pressing. The juice was preserved by freezing until use in laboratory research to obtain ethanol. The resulted bagasse was dried to prevent the proliferation of microorganisms and was stored at room temperature until use. The main steps of the process to obtain second-generation ethanol from sorghum bagasse are: mechanical and physical-chemical pretreatment; enzymatic hydrolysis of lignocellulosic material and fermentation of sugars released from the lignocellulosic complex in the phase of the hydrolysis. The enzymes used in the hydrolysis step can be obtained in situ by cellulolytic microorganisms cultivated on

sorghum bagasse as growth substrate. In this regard, we have established submerged liquid fermentations (SLF) and solid substrate cultures (SSC) of *Trichoderma* and *Aspergillus* fungi. It has been determined endoglucanase and cellulase activities of the extracts obtained from fungal cultures grown on sorghum bagasse and good results have been obtained regarding the enzyme titres, especially in SSC system. In SSC biosystem, the obtained enzyme extracts have higher activity than enzymes obtained in SLF system. The results obtained in this research have been published in the paper ***Sorghum bagasse as substrate for cellulase production by submerged and solid-state cultures of Trichoderma,*** authors Daniela Vintila, Kornel Kovacs, Teodor Vintila, in Scientific Papers: Animal Science and Biotechnologies, 2014, 47 (1) (http://spasb.ro/index.php/spasb/article/view/1776) – volume indexed in international data bases.

Further, sorghum bagasse and other types of lignocellulosic biomass has been subjected to physical-chemical treatment process, followed by enzymatic hydrolysis using fungal cellulases. The results were presented at the international symposium organized by the Faculty of Animal Science and Biotechnology of the USAMVB Timisoara and published in the paper ***Fermentable sugars by steam/alkaline pretreatment and enzymatic hydrolysis of biomass***, authors Adrian Trulea, Horia Barbu, Benoni Lixandru, Daniela Vintila, Georgeta Pop, Teodor Vintila, Kornel Kovacs, in Abstract Book of Animal Science and Biotechnologies Symposium, 2014. Other findings from these studies were presented as a poster at the European Congress of Biotechnology, held in July 2014 in Edinburgh, Scotland. The results are published in ***Enhancing energy yield through saccharification of sorghum bagasse and second generation bio-ethanol production***, authors: Teodor Vintilă, Adrian Trulea, Daniela Vintilă, Georgeta Pop, Iosif Gergen, Kornel Kovacs, in ***New Biotechnology – Volume 31S***, July 2014 (volume indexed ISI, impact factor 2,106). In our study, we used six commercial products containing biomass degrading enzymes to catalyze the hydrolysis of cellulose obtained by pretreating sorghum bagasse and obtain fermentable sugars. Batches were constructed containing combinations of mechanical or chemical-physical (steam-alkali) pretreated bagasse, and various enzyme cocktails. Results indicated combination of bagasse physicochemical pretreated and cellulolytic complex NS22086 (Novozymes) as the most effective. In the next phase, these conditions were applied for hydrolysis of three types of bagasse of Sorghum bicolor (Sugargraze, Jumbo and Fundulea FT132). The percentages of hydrolysis rates obtained for the three types of sorghum where between 32% and 40%. The concentration of reducing sugar and glucose released was monitored in the hydrolysis buffer. The process of conversion continued with the fermentation of obtained hydrolyzates by using *Saccharomyces cerevisiae* yeast in fermenters of 500 ml equipped with a NIR sensor (BlueSens) to assess in real time the concentration of ethanol, oxygen and CO_2. The concentrations of ethanol obtained in fermentation media containing bagasse hydrolyzates from the three types of sorghum were between 1.65 $g \bullet ml^{-1}$ and 1.96 $g \bullet ml^{-1}$. These results indicate ethanol yields between 330 $g \bullet g^{-1}$ and 392 $g \bullet g^{-1}$ reported to dry

weight bagasse. The application of biotechnology developed in this study, in combination with current methods applied to the extraction of sugar from sorghum, will increase the efficiency of ethanol production and total energy productivity will increase. In the perspective on research in biogas from biomass and fermentation residues, it has been developed an automated pilot biogas plant. For this technology a patent application was filed and published in OSIM bulletin: Patent Application no. a 201300067, published in BOPI no. 7/2013, title of the invention: Automated Installation for Biogas Production. Author: Teodor Vintilă.