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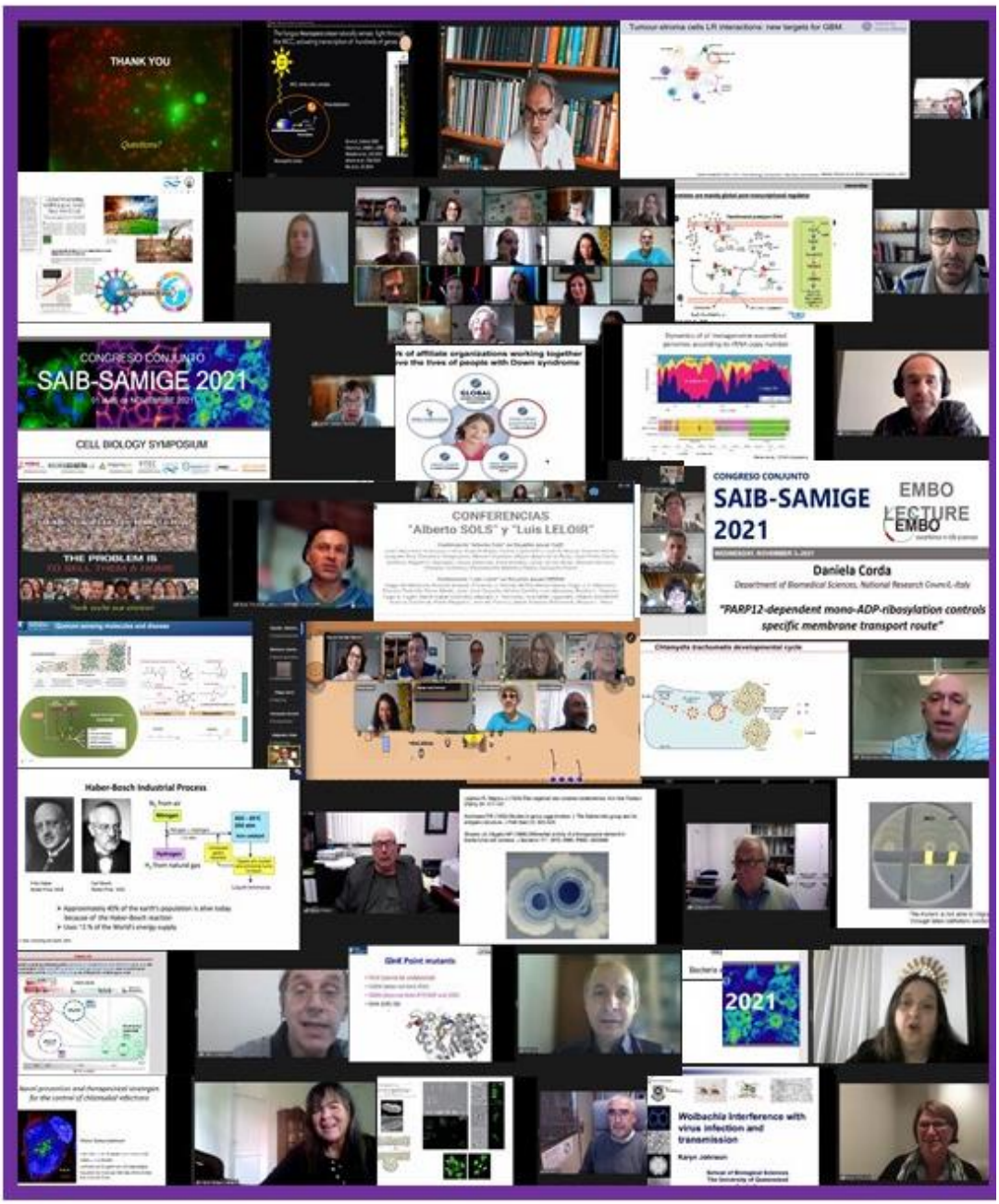
SAIB - SAMIGE Joint meeting
2021 *on line*



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LVII Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

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larger air area. Q breads showed higher polyphenols (as determined by Folin method) and FRAP values, whereas BW breads had significantly higher ABTS values ($p<0.05$). Total starch hydrolysis varied between 38.84% and 72.61%, with a significant reduction observed when sourdough was used. For breads with Q-SD, this reduction was between 34% and 42% compared to the control; whereas it was between 8 and 25% for BW-SD breads. Chemically acidified quinoa breads (Q-ChA) also showed a reduction in starch hydrolysis, whilst BW-ChA bread showed higher starch hydrolysis compared to control and SD breads. In conclusion, SD application in GF systems led to breads of higher technological quality as shown by higher SBV, crumb structure and longer freshness. SD processing also modified starch *in vitro* hydrolysis and antioxidant capacity. Even though these effects seemed to be influenced by pH, a biological effect was also observed.

BT-P11-176
POINT MUTATIONS IN *Echinochloa colona* BIOTYPES CONFER VARIABLE SUSCEPTIBILITY TO GLYPHOSATE HERBICIDE

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Herbicide resistance is an evolutionary and ecological process. The mechanisms involved can be classified in target site resistance (TSR) and non-target site (NTSR), where TSR frequently involves mutations in genes encoding the protein targets of herbicides. Regarding their chemical control, worldwide failures are registered in different populations of *Echinochloa colona*. In Argentina, variable susceptibility to glyphosate was found in some *E. colona* populations, but the levels of susceptibility and mechanism underlying this variation are still unclear. Given that, we analyzed 4 populations of *E. colona* native from central region of Argentina to test resistance levels to glyphosate and molecular variation in the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Regarding to dose-response experiments we found that, between different populations analyzed, the Ec03 population was the most sensitive to glyphosate herbicide. Given that, we established Ec03 population as a susceptible pattern. Using Ec03 as a reference, the glyphosate rate should be increased 3.5 times in Ec04 to produce the same effect of reducing the biomass in 50%, while Ec02 and Ec05 requires to increase only 2.1 times the herbicide concentration to produce the same effect. In survival assays testing glyphosate, population Ec03 did not show resistance, populations Ec02 and Ec05 presented resistance in development, and Ec04 showed survival between 89 and 98% of individuals at the used dose of glyphosate; so that, Ec04 was determined as resistant population. We then proceeded to molecular characterization, specifically focusing in EPSPS mutations. It is known that EPSPS mutations in residues 102 and/or 106 could be involved in glyphosate resistance, so that we performed genomic DNA extraction, followed by PCR and sequencing. Alignment analyses showed that the sensitive population presented the native CCA (Pro) sequence in position 106, whereas semi-resistant Ec02 and Ec05 populations had a mutated ACA (Tyr) sequence at that position in one of the two copies of the EPSPS gene. Taken together, our results suggest that one of the mechanisms underlying *E. colona* resistance/susceptibility in Argentina is TSR type, specifically based in an EPSPS point mutation in position 106.

BT-P12-184
ARABIDOPSIS EXPRESSING A CYANOBACTERIAL NITRIC OXIDE SYNTHASE INCREASED YIELD EVEN UNDER UV-B EXPOSURE

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The enzyme nitric oxide synthase (NOS) catalyzes the biosynthesis of nitric oxide (NO) and citrulline from the substrate arginine. The NOS from the cyanobacteria *Synechococcuss* PCC 7335 (SyNOS) possesses the oxygenase and reductase domains like animal NOS, but it has an extra domain encoding a globin at the N terminus. *In vitro* assays demonstrated that the globin domain of SyNOS acts as a NO dioxygenase, oxidizing the NO to nitrate. As a result, SyNOS is able to produce NO from arginine and also to oxidize it to nitrate with a release rate 75:25 (nitrate:NO). Thus, our hypothesis is that SyNOS expression in plants may allow a greater remobilization of internal nitrogen (N), improving growth and yield. Furthermore, both nitrate and NO produced by SyNOS may positively affect the signaling of various growth processes and/or responses to stresses. Recently, we showed that heterologous expression of SyNOS in Arabidopsis improves N use efficiency, N-deficiency tolerance and yield. In this work, we evaluate the response of SyNOS-expressing Arabidopsis plants to UV-B exposure. Our results show that UV induces morphological changes in UV acclimated plants (exposed to UV-B 1.1 $W.m^{-2}$, 2 h per day during 14 days) which include decreased rosette diameter, decreased inflorescence height, increased numbers of flowering stems and decreased numbers of secondary branches. These UV-induced morphological changes were observed in both transgenic SyNOS and *Rdr-6* control plants. However, the transgenic lines presented increased shoot branching and seed production compared to *Rdr-6* plants in both conditions (with and without UV-B exposure). Chronic UV-B radiation did not affect flavonoid pigment levels, cell damage or ROS production, indicating that UV-acclimated plants were not stressed. Additionally, the effect of prolonged UV-B exposure (irradiated with UV-B 0.5 $W.m^{-2}$ during 6 days) was analyzed in seedlings grown in nutrient agar medium with high (+N, 9 mM NO_3^-) and low N (-N, 0.5 mM NO_3^-) conditions. UV-B irradiation as well as -N condition inhibited hypocotyl elongation in all lines. In -N condition without UV-B SyNOS lines elongated more

the hypocotyl compared to control plants. Under UV-B exposure, increased hypocotyl elongation of transgenic lines was observed only in +N condition. Further investigation is necessary to understand the UV-B acclimation response of transgenic SyNOS lines. Acclimation to a state of stress combination has been shown to involve integrating responses to each of the individual stresses that simultaneously impact the plant (e.g., low N or UV-B stress), as well as the induction of a new type of response, sometimes involving thousands of transcripts, that is unique to the state of stress combination. In summary our results are encouraging towards obtaining crops with better yield under combined stressful conditions.

BT-P13-188
CELL FREE BIOSENSORS FOR DETECTION OF CONTAMINANTS IN WATERS OF THE MATANZA RIACHUELO BASIN

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Water quality assessment is an issue of global relevance. Locally, 15% of Argentina's population inhabits the Matanza-Riachuelo Basin, whose natural and superficial waters have been found not to be apt for human use or consumption due to the presence of natural (ie. arsenic) or anthropogenic (ie. transition metals) pollutants. Monitoring the presence of these contaminants along the basin by local authorities is limited due to the need of specific, expensive, and non-transportable equipment. Recent advances in cell-free synthetic biology have spurred the development of *in vitro* molecular diagnostics. Here, we present the work done in fine-tuning two cell-free biosensors. Both systems can be freeze dried for easy storage and distribution making them a perfect choice as inexpensive point of use water quality assessment devices. First, we report the advances done on a cell-free *in vitro* transcription platform, aptly named ROSALIND. This is a modular system that combines a highly processive RNA polymerase, allosteric transcription factors and synthetic DNA transcription templates to regulate the synthesis of a fluorescence-activating RNA aptamer in presence of a target contaminant. The platform has been validated to detect a range of water contaminants. Here we focus on our recent work of tuning the reaction to detect relevant pollutants in the basin in collaboration with the local authority ACUMAR. More recently, we are working to incorporate a new generation of biosensors based on cell-free protein expression on bacterial lysates that will allow us to widen the range of contaminants we can detect to other common ones such as arsenic and nitrates. Cell lysates-based biosensors allow us to forgo the need to add purified components, decreasing the price and labor cost of individual reactions. The signal can be easily amplified thus allowing for low detection limits. Here we present the first steps on preparing the cell extracts and validating their quality for later use in sensors.

BT-P14-203
EXTREMOPHILE CYANOBACTERIA: *IN VITRO* PRODUCTION OF PROTECTIVE COMPOUNDS AGAINST UVB RADIATION

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The Andean Altiplano-Puna is a sedimentary volcanic plateau at an average altitude of 4000 m located between latitudes 13° and 27° south. Solar irradiance is 165% higher than that at the level of the sea with instant flow of UV-B that reaches 17 Wm^{-2}, low nutrient concentration particularly phosphorous; presence of heavy metals and arsenic and broad fluctuation of the temperature of the air, ranging from 20 °C during the day to -10 ° C at night. Even though these conditions are highly limiting, previous results from our laboratory have described the microbial diversity of different lakes, mats and crust of the altiplano and revealed an unexpectedly diverse microbial community, including several genera of cyanobacteria. Cyanobacteria isolated from extreme conditions like Andean microbial mats and crust could produce mycosporine-like amino acids (MAAs) as a mitigation strategy to reduce the damaging effect of ultraviolet radiation. In order to probe the production of MAAs from these cyanobacteria, the analysis of the methanolic extract by spectrophotometry was used as a rapid method to know the presence or absence of these compounds. Different strains of cyanobacteria were placed in quartz tubes and exposed to UVB radiation for 4 and 24 hours. Then the culture was centrifuged at 10000 rpm during 10 min, and 0,15 g of biomass were placed in tubes with 15 ml of methanol during 24 h in dark. Methanolic extract was analyzed in a spectrophotometer by scanning from 250 nm to 750 nm. Also, the methanolic extract was analyzed with high performance liquid chromatographic (HPLC), using Waters Alliance 2695e - Waters PDA 2998 Detector - Empower 2 Software, Column: Gemini C18-5u-4.6 x 250 mm and the mobile phase: 0.1% acetic acid in methanol 2.5%. The cyanobacteria that showed presence of MAAs were cultivated in different conditions in order to determine the most convenient: condition 1: light 24 h, without shaking and room temperature; condition 2: light:dark 12:12 h, without shaking and 28°C; condition 3: light:dark 12:12 h, without shaking but bubbling air into the reactor and 28° C. The strain GTAR 001, *Anabaena sp*., showed the most significant peak of absorbance at 334 nm when was exposed for 24 h to UVB. The MAAs reported for cyanobacteria have peaks between 310 and 360 nm and the peak of 334 nm corresponds to Shinorine. On the other hand, the strain GTAR 001 produced significantly more biomass with condition 3 and in a shorter period. These preliminary results agree with the MAAs reported for *Anabaena sp.* isolated from rice paddy field and hyper saline pond/marine habitat. It will be necessary to analyze this compound with liquid chromatography coupled with tandem mass spectrometry for the final determination.