Physiological, ionomic and metabolomic profiling of Szarvasi-1 energy grass under limiting and stimulating conditions (K132241)

Final report

The project aimed to reveal the ionomic properties of the Szarvasi-1 energy grass (*Elymus elongatus* subsp. *ponticus* cv Szarvasi-1), a large biomass plant frequently used for biogas production, recultivation of contaminated lands or waste dumps and potentially used for forage in animal husbandry. The plants were grown on heavy metal/trace element contaminated substrates and substrates of high salt content. We have applied stimulatory or protective treatments and tested the plants resilience. We consider the project successful in terms of scientific goals although there were several hindering events. These included the Covid pandemic, energy crises and the sudden breakdown followed by a slow repair of the XRF imaging instrument in the core facility. Finally, five Q1 (including 2 D1) papers have been published and there is one more manuscript not yet submitted.

Objective 1. ***Salinity stress tolerance of Szarvasi-1 energy grass***

Over the biomass plant Szarvasi-1 energy grass we included giant (energy) reed (*Arundo donax* cv Blossom) (another biomass plant) and wheat (*Triticum aestivum* MV Nemere) (agricultural crop) to the investigation of the effect of salinity stress. The experimental design included the application of NaCl in nutrient solution in climate chamber and $NaHCO_3$ + Na_2CO_3 in soil cultures in glass house conditions. The open-air container salinity experiments has been changed compared to the original plan. Due to technical difficulties in handling large amount of soil amended with NaCl and in order to improve the study / to achieve higher ranked results we shifted the focus towards the understanding of the molecular biological backgrounds. We have finally published two papers in these topics (Müller et al, 2022; Arcoverde Cerveira Sterner et al., 2024). Szarvasi-1 was also characterised for its pH and nitrate:ammonium preference.

Effect of salinity (NaCl) in Szarvasi-1 plants

(Arcoverde Cerveira Sterner et al., 2024)

The effect of high NaCl concentration (0, 50, 100, 200 mM) was comparatively studied in Szarvasi-1 energy grass and wheat. A culturing procedure has been established that involved a pregrowth of plants in a nursery of 10 L containers and then a transfer to 0.8 L pots each containing 3 plants followed by the two week time treatments in each experiment. The shoot dry weight significantly decreased in both cultivars only at the highest NaCl concentration. In wheat, 50 mM NaCl caused a slight increase. But the pH of the nutrient solution showed a larger decrease in wheat along with increasing NaCl. The stomatal conductance of the youngest fully developed leaves continuously decreased with the increasing NaCl concentration and this was represented by a significant linear fit on the second week of treatment. Energy grass showed a higher water saturation deficit (7-9%) and it was not changed by the treatments while for wheat it increased from 4.5% to 9.9%. The water potential of energy grass leaves was an order of magnitude higher than that of wheat plants. Nevertheless, it decreased significantly with increasing NaCl concentration and treatment time. The maximal quantum yield of PSII (Fv/Fm) in energy grass continuously decreased with increasing NaCl but the malondialdehyde

(MDA) content was not different in roots and it was even lower in shoots as compared to the untreated control.

For the ionomics analysis of Szarvasi-1 in comparison with wheat the measurements were made with ICP-OES. Principal component analysis was conducted on all data obtained. In all PCA biplots a strong negative correlation between Na and K was found in both plants but Szarvasi-1 maintains a lower Na/K ratio under excessive Na exposure. Water potential, RWC, Fv/Fm, stomatal conductance, fresh and dry weight data show a negative correlation with the Na vector. MDA had a positive correlation with Na in wheat but not in Szarvasi-1. Fe, Zn and Cu uptake are not affected by salinity stress in either plants. The Ca, P, Mg, K, Mn and S translocation to shoot is severely disrupted by salinity stress. Mo uptake had a positive correlation with Na in both shoots and roots of Szarvasi-1 and wheat.

We performed a large-scale expression analysis study on the Szarvasi-1 plants using the same hydroponics conditions. Results showed that phenylpropanoids play an important role during early and late stress response as well, depending on the extent of salinity. The same conclusion can be made in the case of ion transporters such as SOS1 (Salt Overly Sensitive 1). The members of the photochemical apparatus and photorespiration show a highly varying expression pattern depending on time and salt concentration, yet it is obvious that they are taking part in the stress response from the very beginning. In the shoot, the control group had a positive correlation with MDA concentration and most of the elements except for Mo, Cu, and Fe. Among genes, *Dihydroflavonol 4-reductase (DFR)* had the strongest relationship with the control group but *Cellulose Synthase A catalytic subunit 1* (*CESA1*), *CP43* (*PSBC*), and *Mitogen-activated Protein Kinase 6* (*MAPK6*) also correlated well with this group. Increasing the NaCl concentration to 50 mM there is a transition in the correlation of physiological parameters (fresh and dry weight, water potential, stomatal conductance, maximal quantum efficiency of PSII) to the Na50 group. The genes *Sodium Proton Exchanger 1* (*NHX1)* and *Serine Hydroxymethyltransferase 1* (*SHM1*) showed a very significant positive correlation with this group. The Na100 treatment group correlated positively with *SOS1*, *Cinnamyl Alcohol Dehydrogenase 5* (*CAD5*), *Pheophorbide a Oxygenase* (*PAO*), and *Heat Shock 70 kDa* (*HSP70*) whereas the Na200 group was closely correlated with *Chalcone Synthase (CHS*), *Oxygen-evolving Enhancer 1* (*PSBO*), *D2* (*PSBD*), and *Heat Stress Transcription Factor A-1 (HSFA1)* but showed a good correlation with *HSP70* as well. HSP70 is a heat sock protein and a positive regulator of SOS1 Na/H^+ exchanger while HSFA1 is a transcription factor regulating the response of plants to various abiotic stresses. CESA1 is the catalytic subunit of the cellulose synthase enzyme the altered expression of which may have a role in stress response at the level of cell anatomy. Overall, it seems that Szarvasi-1 has a well-functioning strategy and cellular apparatus in order to alleviate salinity stress.

pH and nitrogene form preference of Szarvasi-1 energy grass

Although energy grass is thought to be tolerant to alkaline pH soils, in hydroponic cultures it showed a clear physiological optimum at pH7 when the plants were grown in buffered solutions with initial pH values between 4 and 9. This was observed from root and shoot weight, root relative water content (RWC), chlorophyll a+b concentration of the leaves and the stomatal conductance. The effect of different nitrate to ammonium ratios were studied by a stepwise change in the nutrient solution (NO3:NH4 %: 100:0, 50:50, 25:75, 10:90, 1:99, 0:100). Based on the root and shoot dry weight, the 25:75% ratio seemed to be optimal with lower values below and above that ratio. However, the physiological parameters (solution pH, transpiration, Chl a+b, Chl a/b) do not justify the positive effect of increasing ammonium supply compared to sole nitrate nutrition.

Alkaline salt stress in Szarvasi-1, wheat and energy reed

(Müller et al, 2022)

The effect of high soil salinity was investigated on Szarvasi-1 energy grass, in comparison with wheat and energy reed. After one month pregrowth, salinity and alkaline stress was induced by irrigation with $NaHCO_3$:Na2CO$_3$ 1:1 buffer at two salt concentrations - 80 (medium) and 200 mM (high) - while the control plants were irrigated by deionized water only.

Salt stress tolerance was analysed based on the photosynthetic activity as well as by the monitoring the antioxidative defence status by total SOD enzyme activity measurements. According to the photosynthetic activity measurement (measured by LiCOR 6800F) energy grass is the most tolerant to salinity stress since no significant difference was observed in transpiration, CO_2 absorption, the maximal and actual quantum yield of PSII or non-photochemical quenching (NPQ), regardless of the salt concentration. The energy reed tolerated the medium stress while high salt concentration caused severe decrease in all these parameters, except NPQ which increased. Even at medium salinity stress the photosynthetic parameters of wheat changed remarkably compared to the control indicating that wheat is less tolerant to the applied salinity stress treatment than the energy reed and energy grass. Based on the SOD activity measurement we found that in Szarvasi-1 the oxidative stress caused by salt stress was lower than in the other two species as the total SOD activity in treated plants was similar to that of the control while in the energy reed and wheat the total SOD activity considerably increased at 200 mM and interestingly at 80 mM it decreased slightly but not significantly. In giant reed, MDA content of the leaf was increased by 7.7% and 12.3% at 80 mM and 200 mM $NaHCO_3$ + Na_2CO_3, respectively, compared to the control. Total superoxide dismutase (SOD) and Class III peroxidase (POD) activity, was increased by 24.1% and 27.2%, and 7.5% and 42.7 at 80 mM and 200 mM, respectively, compared to the control. Similarly, glutathione reductase (GR) activity increased by 9.9% and 21.1% and catalase (CAT) activity by 17.7% 5.7%. Ascorbate peroxidase (APX) I activity gradually decreased with the increasing salt stress but the change became significant only at 200 mM salt. In APX II activity an opposite tendency was detected where the increasing concentration of salt resulted in higher enzyme activity especially at 200 mM. Ascorbate oxidase activity significantly decreased at both applied salt concentrations. Na/K ratio was maintained at 80 mM salt concentration but significantly increased at 200 mM.

Objective 2. *Effect of different amendments on the growth and physiological parameters of Szarvasi-1*

In this topic we have grown Szarvasi-1 plants on various substrates as nutrient solution amended with red mud, Zn, Cu, Cd and Pb, and soils collected in Budatétény, Gyöngyösoroszi, the Zemplén mountains, at Salgótarján which were differently contaminated with Pb, Cu, Zn, Cd or As. We also investigated the effect of sewage sludge applications in open air soil cultures. The experiments aimed to reveal the physiological limitations and element accumulation capacity of Szarvasi-1 plants. We have published two papers so far (Kolberg et al., 2022; Rana et al., 2025).

Effect of red mud on growth and physiological parameters and elemental composition of Szarvasi-1 plants

Red mud is a hazardous material but may have potential uses in industry and agriculture depending on its composition. Red mud sample originating from Almásfüzítő, Hungary was used in an experiment to evaluate its toxic effects or nutritive value. Its major constituents included Al, Cr, Ni, and Cu. The toxicity was studied with Szarvasi-1 energy grass grown in unfiltered ¼ Hoagland solution spiked with 0, 1, 5 and 10 g red mud. There was no significant change in the dry shoot matter but on the contrary, the root growth was stimulated by all treatments up to 10 g. While shoot water content was slightly decreased, root water content increased by 10%. Transpiration and Chl a+b decreased over 1 g red mud treatment. When the nutritive value of red mud was investigated the dry shoot and root mass decreased slightly at 1 and 5 g treatment. But while the root water content continuously increased with increasing red mud addition the shoot water content decreased only at 10 g red mud. Transpiration and Chl a+b decreased by the treatments. The concentration of elements was measured and compared only at 5 g red mud treatment. All nutrient ion concentrations decreased except for Na in the roots and shoots as compared to the control in the plant grown in ¼ Hoagland. In 1/40 Hoagland only the Mg decreased in the roots but Ca, Fe and Cu decreased in the shoots. The rest of the nutrients increased. We have found no Al, Cr and Ni accumulation in the shoot at any of the applied treatments.

We have concluded that the investigated red mud sample proved to be a relatively neutral material in terms of its effect on Szarvasi1 energy grass.

Effect of sewage sludge on growth and physiological parameters and elemental composition of Szarvasi-1 plants

12 fully closed plastic containers of 0.2 m^3 volume were installed in the Huzella Garden – Botanical Garden, Eötvös Loránd University, Göd. The containers have been filled up with low nutrient sandy soil (pH 7.41, KA <25, Humus w/w 1.07%), filtered and homogenised properly. The nutrient content of the soil has also been determined. The germination was ensured by regular irrigation. The plants have developed tillers but not flowering stems during 2021 as expected. The containers have been treated with communal sewage sludge obtained from the Budapest Sewage Works Pte Ltd. in spring 2022. The treatments were 0, 1, 1.5, 2 kg sludge / 200 dm3 pot in three parallel. The dry total biomass / pot almost doubled by 1 and 1.5 kg sludge while the largest dose increased it by 125% in the summer. The autumn yield, which is usually smaller (it was half of the summer yield) increased to 198, 237 and 290 % of the control by 1, 1.5, 2 kg sludge, respectively. Interestingly, the stomatal conductance was only 15-18% of the control grasses in all treatments while the chlorophyll content (SPAD index) was 125% of the control in the 1 kg sludge treatment, 138% in the 1.5 kg treatment above which it did not increase in the 2 kg treatment. The maximal quantum efficiency of photosystem II showed that the control and 1 kg sewage sludge treatments were slightly stressed but at 1.5 kg sludge the Fv/Fm reached healthy level (0.79) which confirms that the soil was nutrient poor and the sludge applied was indeed necessary to supply the adequate amount of nutrients.

The macroelement and Na content that could be measured in the collected tissues did not differ markedly in the different treatments. P showed a decreasing trend compared to the control in the case of increasing sewage sludge doses, in the samples collected in May. Nitrogen showed an increasing trend. It is also worth mentioning the significant increase in Na in the S2 and S3 doses, and in the sulphur in the S1 dose in the May sample. At the same time, these outstanding data cannot be explained by the plant's increased ion uptake, since a similar effect should have been seen in the case of other treatments. In the case of N, Mg, Fe and Mn, the tissue concentration also increased markedly in the autumn samples. These elements play an important role in the construction of the photosynthetic apparatus. Their increased concentration is a condition for rapid growth and higher chlorophyll content. Although the former prevailed in the biomass yield, the parameters characteristic of the photosynthetic apparatus, such as the Chl content, did not exceed the spring values. Thus, the accumulation of the four elements would presumably have served to increase the biomass left in the longer autumn period. Compared to the control, Zn and Cu also showed a small increase with the sewage sludge treatments.

We also rented 48 closed, 1 m^3 open air containers at the Agricultural Research and Development Institute (ARDI). The sewage sludge experiments have been repeated on already developed Szarvasi-1 cultures. The measurements indicated very similar effects to those measured in Göd. In these plants the MDA content was also followed and it increased significantly only by the highest sludge treatment (in the autumn sampling) to 145% of the control. The dry biomass contained a markedly increased amount of N, K and Ca. Na also increased.

Zinc accumulation and tolerance in Szarvasi-1 pants

(Kolberg et al., 2022)

As in previous studies Szarvasi-1 was proved to be tolerant to high Zn concentration, zinc excess nutrient solution experiment was carried out under lab conditions to reveal its accumulation capacity. In this treatment solution, zinc was added as surplus $ZnSO_4 \cdot 7H_2O$ in 0 (control), 0.01, 0.05, 0.1, 0.5 and 1 mM concentration above the 0.19 µM Zn already applied in the basic solution. Biomass, physiological parameters and ionomic composition was measured. The root and shoot weight, shoot length, shoot water content and stomatal conductance proved to be only sensitive to the highest applied Zn concentrations, whereas the concentration of MDA increased only at the application of 1 mM Zn in the leaves. Although physio-logical status proved to be hardy against Zn exposure, shoot Zn content significantly increased in parallel with the applied Zn treatment, reaching the highest Zn concentration at 1.9 mg g-1 dry weight. The concentration of K, Mg and P considerably decreased in the shoot at the highest Zn exposures where that of K and P also correlated with decrease in water content. Although the majority of microelements remained unaffected, Mn decreased in the root and Fe content was in a negative correlation with Zn both in the shoot and root. In turn, application of excessive EDTA maintained a proper Fe supply for the plants but lowered Zn accumulation both in roots and shoots. Thus, the Fe-Zn competition for Fe chelating phytosiderophores and/or for root uptake transporters fundamentally affects the Zn accumulation properties of Szarvasi-1. Indeed, the considerable Zn tolerance of Szarvasi-1 has a high potential in Zn accumulation.

Effect of trace elements in soil samples collected in contaminated sites

Soil cultures were established in the glass house to investigate the effect of heavy metals and As on Szarvasi-1. The soils were collected at the former industrial site of the Metallochemia company in Budatétény (MET), at Gyöngyösoroszi (mine dump, GMD and forest, GF), in the Zemplén region (differently As contaminated sites, ZAs1, ZAs2) and at Salgótarján (slag dump, SSD). Uncontaminated sandy soil from Budapest garden area was used as control. The element concentrations were determined in the soil substrates and the following elements exceeded the levels allowed in soils: MET: Pb > Zn > Cu > Cd; GMD: Pb > Zn > Cu > Cd > As; GF: Zn > Pb > Cu; ZAs1 < ZAs2: As > SSD: As. The soils were moistened and kept at 70% field capacity and the seedlings were grown for 45 days in greenhouse conditions. Shoot dry matter yield decreased in the following order: GF > control >> SSD > ZAs1 > GMD, ZAs2 > MET. “>>” means more than 60% growth inhibition. The stomatal conductance of the first fully developed leaves showed two levels: GF, control, SSD > ZAs1, ZAs2, GMD, MET. Interestingly the Chl a+b concentration has not been affected significantly only at MET and GMD. The shoot As concentration was very low and in most cases remained below detection limit. Heavy metal accumulation was found in the shoots in the following order in general: Zn > Pb > Cu > Cd. However, the accumulation was dependent on the concentration of element in the soil: in MET the accumulation order was: Pb > Zn > Cu > Cd. Nevertheless, the poor growth of the plants in highly contaminated soils did not allow sufficient number of measurements and the repetition of the experiment is not possible due to the limited soil sample supply. For this reason we have started a new experimental set with a sandy garden soil artificially amended with heavy metals.

Effect of trace elements in artificially amended soil in single and combined treatments

(Rana et al., 2025)

We have developed the experiments with soils amended with different heavy metals. Copper (100, 500, 1000 mg/kg soil), cadmium (5, 10, 50 mg/kg soil), lead (100, 500, 1000 mg/kg soil) and combined treatments Cd5Cu100Pb100 (series1), Cd10Cu500Pb500 (series2) and Cd50Cu1000Pb1000 (series3) were applied in sandy soil and physiological parameters as well as total shoot element content was measured. The dry weight decreased most significantly under Cd treatment to 20% of the control while under Cu treatment the decrease was 50% and under Pb treatment it did not change. Relative water content decreased with increasing Cu concentration but it did not change with Cd and Pb. The decrease in chlorophyll concentration followed the order Pb (9%) << Cd (67%) < Cu (75%) (% in brackets shows the decrease at the highest concentration). The photochemical reflectance index (PRI) showed that the plants were very sensitive to Cu treatment and its values reached the negative range at 1000 mg/kg Cu. Under Cd treatments PRI also decreased but remained in the positive range while under Pb exposure it did not change. In the combined treatments the growth variables, Chl and PRI declined rapidly and MDA increased showing the exhaustion of detoxification capacity of the plant. The accumulation of heavy metals (bioconcentration factor) decreased in the following order Cd>Cu>Pb in both single and combined treatments but in the latter the accumulation pattern slightly changed in favour of Pb. The nutrient and trace elements showed a characteristic change in their pattern in the shoots.

We have performed several multivariate analyses to find correlations and identify characteristic changes in the ionomic patterns of the plants. The PCA (Principal Component Analysis) results indicated a negative correlation between Cd-treated and Ctrl plant groups, with a strong positive correlation with Cd observed at Cd50, suggesting increased Cd uptake by plants when exposed to the highest Cd concentration. Interestingly, Fe showed a slight correlation with Cd5 plants, but not as pronounced. PCA showed that Ctrl plants are well separated from the Cu treated plant groups suggesting that different concentrations of Cu have a strong impact on the mineral uptake pattern of energy grass. The majority of the mineral elements such as Mg, Cu, Ca, Fe, Na, S, Cd, Ba, and Zn showed a positive correlation with Cu500 and Cu1000 plants groups suggesting that Cu treatment at these concentrations has increased the uptake of mineral elements from the soil. Interestingly, Fe and S are strongly correlated with Cu1000. In the PCA of Pb-treated plants, Ctrl plants clustered closer to Pb100 plants, suggesting a lesser impact of Pb at this concentration. Conversely, plants exposed to the highest Pb concentration (Pb1000) showed a stronger correlation with Pb, indicating higher uptake by energy grass plants. Mineral elements such as Mg, Mn, Cd, K, Na, Zn, S, and Fe exhibited positive correlations with both Ctrl and Pb100 plants, suggesting optimal mineral uptake in Ctrl and altered patterns at higher Pb concentrations. However, elements such as Ca, Ba, and Cu showed higher correlations with the Pb1000 plant group, suggesting their better uptake at this concentration. In the PCA plot, Series 2 and 3 are placed at a considerable distance and are well separated from Ctrl and Series 1 suggesting variations in their mineral uptake pattern among all groups. However, Ctrl and Series 1 were found closer to each other. Mineral elements such as Mn and Ba lean more towards Series 2 while K showed a higher correlation with Ctrl and Series 1. Na also leans towards Ctrl plants. Also, Cu, Cd, and Pb, lean more towards Series 2 and 3 suggesting their concentration-based uptake.

The LDA (Linear Discriminant Analysis) graph showed that Ctrl and Cd5 plants are relatively closer together, indicating that a Cd5 has a lower effect on mineral uptake. However, minerals such as Mn, Mg, Cu, Ca, S, Na and Fe showed a higher correlation with Cd5 plants compared to Ctrl plants. Cd10 and Cd50 plants were nicely separated from Ctrl and Cd5 plants, and most of the other mineral elements clearly also indicate a negative correlation with these two groups. Additionally, Cd represented a high correlation with Cd50 plants. LDA graph of Cu-treated plants represented that all the groups are well separated from each other suggesting differences in their mineral uptake patterns due to the exposure of plants to Cu. Elements such as Mg, Ca, Cu, Mn, and Na indicated a positive correlation with the Cu500 plants. Cu1000 plants are placed very far from the other groups plotted, suggesting a higher degree of separation and alteration in patterns of their mineral uptake at this particular concentration. Our results indicated that Ctrl and Pb100 plants overlapped with each other, suggesting similar patterns of their mineral uptake process. In contrast, there was a noticeable difference in Pb500 and Pb1000 plants, which are in complete contrast to Ctrl and Pb100 plants. Mineral elements such as Na, Fe, Mn, S, P, Mg, and Zn showed a positive correlation with Ctrl plants and account for a considerable distance from Pb500 and Pb1000, suggesting a considerable impact of higher Pb concentrations on the mineral uptake. It was clear, that Pb uptake was higher in group Pb1000. Interestingly, minerals such as Cu, Ba, and K are placed between the Ctrl and Pb1000 plants, somehow suggesting that their uptake is not influenced by the Pb concentrations in the soil. Similar to PCA, LDA also showed divergence among all four plant groups. Mineral elements such as Ba, Mn, and Mg lean towards Series2. Cu, Pb, and Cd also showed a correlation with plant Series2 and 3.

Objective 3. ***Evaluation of protecting treatments against different types of environmental stress***

We have tested S-methylmethionine (SMM) as priming agent prior Cd stress as an additive to nutrient solution and SMM and Na-salycilate (NaSA) prior drought stress as foliar spray. We have also applied various nanomaterials to rescue Szarvasi-1 plants from iron deficiency. One paper has been published (Rana et al., 2022) and another manuscript has been written but not submitted, yet (Müller et al., 2025).

S-Methylmethionine effectively alleviates stress in Szarvasi-1 energy grass by reducing root-to-shoot Cd translocation

(Rana et al., 2025)

SMM is a non-proteinogenic amino acid that plays an important role in the sulphur metabolism in plants whereas Cd is a highly toxic heavy metal that poses a harmful effect on growth and metabolic pathways in the plants. Szarvasi-1 has shown relevant tolerance to heavy metal stress (Pb, Zn, Ni) but its priming with SMM has not been evaluated so far. Preliminary experiments have been performed in hydroponics to explore if any changes in the physiological parameters could be associated to SMM but we have found no significant affect so we continued with Cd treatment experiments. 24 hour SMM treatment (0.01 mM, 0.05 mM, 0.1 mM) was applied to the plants and 0.01 mM $Cd(NO_3)_2$ was applied after the removal of SMM. Effect of SMM pretreatment has been analysed for different physiological parameters such as Chl *a+b* concentration, photochemical reflectance index, Chl *a* fluorescence, stomatal conductance, RWC. Acclimation of Cd induced oxidative stress was indicated by leaf MDA analysis. Antioxidant enzymes (SOD, POD) activities were analyzed by native PAGE. Growth as well as accumulation pattern of Cd and essential mineral nutrients have been investigated.

Cd exposure decreased the root and shoot growth, Chl concentration, stomatal conductance, photosystem II function and increased carotenoid content. Except for stomatal conductance, SMM priming had positive effect on these parameters compared to Cd treatment without priming. In addition, it significantly decreased the translocation and accumulation of Cd. Cd treatment decreased K, Mg, Mn, Zn and P in the roots and K, S, Cu and Zn in the shoots compared to the untreated control. Based on principal component analysis and canonical analysis of variance, SMM priming changed the pattern of nutrient uptake of which Fe showed characteristic accumulation in the roots to increasing SMM concentrations. We have concluded that SMM priming exerts a positive effect on Cd stressed Szarvasi-1 plants that retained physiological performance and growth. This ameliorative effect is suggested to be based on, at least partly, the lower root-to-shoot Cd translocation by the upregulated Fe uptake and transport.

SMM and NaSA applied as foliar sprays to mitigate drought stress effects

(Müller et al., 2025)

We have established experimental quadrates in the Huzella Garden, Eötvös Loránd University, Göd with newly sown Szarvasi-1 energy grass (50 m^2). Furthermore, we have rented 200 m^2 plough land of previously established Szarvasi-1 energy grass plantation ARDI) for open-field investigations of stress-protective compounds. One square meter quadrats have been marked separated with one meter corridors in all directions. The whole experimental area was managed by the local staff.

Foliar treatments with SMM and NaSA has been applied in the previously established experimental quadrates in the sandy soil of Huzella Garden, Göd, and in the solonchak soil of ARDI, Szarvas, twice in two consecutive years. At both sites severe drought stress has stroke the plantations in 2021 May to July, the main growth period of the plant. Four to seven days after treatments CO_2 fixation (A), the maximal (Fv/Fm) and actual (Φ_{PSII}) quantum efficiency of photosystem II, non-photochemical quenching (NPQ), transpiration (E) and Chl a+b concentration was measured and samples have been collected for ionomics and untargeted metabolomics analysis as well as for enzymatic assays to study antioxidant capacity . At harvest, water content, dry matter yield have been determined. Both SMM and NaSA stimulated E, A, ΦPSII and decreased NPQ. Fv/Fm was increased only by NaSA treatment. However, water content, dry biomass and chlorophyll content was not affected by the treatments.

Enzyme activity assays were conducted to evaluate whether biostimulant treatments influence the antioxidant defence mechanisms of energy grass under drought stress. Due to methodological constraints, these measurements were limited to the Huzella Garden experiment. In NaSA-treated plants, SOD, POD, and GR activities were significantly enhanced, while APX and GPOX activities decreased, accompanied by a declining trend in CAT activity. Similarly, SMM treatment led to increased SOD, POD, and GR activity, whereas APX, CAT, and GPOX activities were reduced compared to untreated controls.

An untargeted metabolomics profiling has been performed in the Agricultural Research Center, HAS and metabolomic data set has been analysed to delve deeper into the effects of these treatments. Data analysis of the metabolomics profiling revealed that applying SMM on plants led to elevated levels of sulphur-related metabolites, like methionine, although the difference observed was not

deemed significant. Among the osmolytes analyzed, the concentration of fructose showed a notable increase, primarily evident in the initial year of observation. Conversely, the second year displayed minimal change in fructose levels, albeit a slight elevation was noted. Meanwhile, glucose, another crucial osmoprotectant, exhibited an increase that contrasted with fructose dynamics. In the first year, glucose accumulation lacked significance, whereas in the subsequent year, glucose content displayed a noteworthy surge, reaching 20-30% higher levels compared to untreated plants. Interestingly, the application of SMM resulted in a drastic reduction of ascorbic acid content during the initial year. The analysis also revealed that in SMM-treated energy grass the amount of the shikimic acid has been increased especially in the second year of the treatment. Although shikimic acid is the known precursor of aromatic amino acids, L-phenylalanine, and L-tyrosine, level of these amino acids has been altered in the reverse direction. The application of SMM has affected the levels of various amino acids. Specifically, valine levels notably decreased in the first year following SMM treatment. Additionally, both L-isoleucine and leucine content exhibited declines in plants treated with SMM. Interestingly, in energy grass plants subjected to SMM treatment, the proline concentration exhibited stability, showing no substantial alterations. The levels of serin and glycine were reduced following SMM treatment over two consecutive years, but a notable difference was observed solely in the first year, where the impact of drought stress was more pronounced compared to the second year. When exposed to SMM treatment alongside drought stress, there has been a significant decrease in the levels of L-aspartic acid over the first and second years. Additionally, both L-glutamic acid and L-asparagine exhibit a trend of declining levels. The TCA cycle also underwent changes due to SMM treatment, resulting in a noticeable alteration in the levels of its intermediary components. Aconitic acid and citric acid notably decreased, especially in the second year. Malonic acid decreased as well, though a significant difference was observed only in the initial year. Succinic acid and fumaric acid, further key TCA cycle constituents, did not exhibit significant changes in plants treated with SMM. Conversely, the levels of malic acid and oxalic acid, both integral to the TCA cycle, increased.
The impact of applying NaSA has shown the influence seen with SMM treatment on the levels of sugars and sugar alcohols. Fructose and glucose has shown increasing tendency as seen in the case of SMM treatment. In NaSA-treated plants, there was an overall increase in the content of malic acid and oxalic acid, both integral to the TCA cycle. Conversely, other TCA intermediates such as aconitic acid, malonic acid, succinic acid and fumaric acid notably decreased in NaSA-treated plants. Overall, while NaSA treatment impacted various TCA cycle compounds, significant alterations were observed selectively across the organic acids studied, delineating a nuanced effect on these intermediates. NaSA treatment caused varying effects on different amino acids over two years. In both years, serine, glycine, L-alanine and L-valine decreased with the NaSA-treatment, but the significant difference was evident only in the first year, which experienced more severe drought stress. Aspartic acid, vital in amino acid metabolism, decreased with NaSA treatment, notably significant only in the second year, along with glutamic acid and asparagine. Conversely, methionine content notably increased in both years in NaSA-treated plants, with an extremly huge, 20-fold rise in the first year and more than double in the second year compared to the control plants suffering from drought stress. Proline concentration remained unchanged, but L-5-oxoproline increased remarkably in the first year. These results demonstrate a complex and varied impact of NaSA treatment on amino acid levels across the two years, with certain amino acids responding differently and exhibiting year-specific changes.

Based on the element content of the shoot tissue samples collected at the same time when samples for metabolomics were taken we have tried to find correlations between elemental patterns and the treatments. However, it was found that the first sampling did not show difference between the treatments. Then the consecutive samples showed increasing positive correlation with SMM treatments and negative correlation with NaSA treatments. This could also be correlated with increasing drought.

The correlations between the data were analysed using PCA. The metabolomic and physiological characteristics of the untreated plants (ctrl) and those treated with SMM and NaSA were well separated in the PCA in both investigated years. In 2021, the SMM treatment caused significant differences compared to the control, indicating a strong effect of the treatment on certain metabolites such as caffeic acid, phenylalanine, and tyrosine. The effect of the NaSA treatment was more moderate, but changes were also observed, for example, in the levels of oxalic acid and glutamic acid. The differences suggest that both treatments elicited different but distinguishable metabolic responses involved in alleviating drought stress.

In 2022, the results showed a similar trend: the effect of the SMM treatment caused unique metabolic responses but the NaSA treatment was more pronounced in the second year. The metabolic patterns of the two treatments were closer to each other, which may indicate the stabilization of the long-term effects of the treatments in the perennial Szarvasi-1. Based on the loading vectors, metabolites such as caffeic acid, phenylalanine and tyrosine were associated with the SMM treatment, while the levels of ascorbic acid and glutamic acid increased in the case of the NaSA treatment. Besides, SMM treatment showed a positive correlation with potassium. The differences emphasize that the two biostimulants improve the ability to withstand stress through different mechanisms.

Effect of two Fe-based nanomaterials on Fe deficient Szarvasi-1 plants

Energy grass plants were grown with optimal and suboptimal Fe supply in hydroponics. Two differently formulated, manufactured nanoZn-ferrite and nanomagnetite materials were added to the nutrient solution. In previous studies it was found that nanoMn-Zn-ferrite and nanomagnetite had a positive effect on model plants and nanomagnetite was shown to be taken up and translocated to the shoot. In our experiment the plants should have been supplied with the Fe-containing nanomaterials after one week of growth as without Fe they showed symptoms of chlorosis and senescence. The treatment was applied in 0.02 mM Fe-containing solutions. After two weeks of further growth, the control (Fe-citrate-treated) plants were growing normally but the nanomaterial-treated plants had uniformly 16% and 25% shoot and root dry weight compared to the control, respectively. The pH of the nutrient solution increased to 6.7-6.8 by nanomagnetite treatments similar to the control but for the nanoZn-ferrite-treated plants it remained at the Fe-deficient level, pH 6.3-6.5. The Chl a+b concentration of the leaves also remained at the Fe deficient level in all nanomaterial-treated plants (1000 microgram/ g FW) while the control ones reached 2800 microgram/ g FW. As the leaves were too small to perform stomatal conductance and fluorescence induction measurements, we have decided to apply a hand-held instrument to measure photochemical reflectance index (PRI) which correlates with carotenoid level and related to the photosynthetic light use efficiency. The PRI values were lowest (in the negative range) in the nanoZn-ferrite-treated plants but they did not differ from the nanomagnetite treated and Fe deficient plants significantly, while the control plants had 0.011 PRI. We have concluded that Szarvasi-1 does not utilize the applied nanomaterials in nutrient solution during a short-term application.

Publications

Thesis

Jobbágy K. (2022) Investigation of gene expressions in Szarvasi-1 energy grass under salinity stress (Szarvasi-1 energiafű génexpressziós vizsgálata sóstressz fennállásakor). MSc thesis, ELTE. SZD21030408159696

López P (2021): Comparative study of energy grass and wheat responses to elevated salt concentration. MSc thesis, ELTE. SZD21030408161010

Conferences

Fodor F, Müller B, Arcoverde Cerveira Sterner V, Rana D, López P, Kolberg F, Tóth B, Rudnóy Sz, Sipos Gy, Solti Á (2021) Szarvasi-1 energiafű – stressz tolerancia, rekultiváció, biomassza produkció. In: Összefoglaló kötet, XIII. Magyar Növénybiológiai Kongresszus, p19. (oral pres. Fodor F)

Arcoverde Cerveira Sterner V, Rana D, López P, Müller B, Tóth B, Sipos Gy, Solti Á, Fodor F (2021) Elemental and physiological correlations between wheat and Szarvasi-1 energy grass under salinity stress. In: Összefoglaló kötet, XIII. Magyar Növénybiológiai Kongresszus, p56. (poster pres. Arcoverde CSV)

Rana D, Arcoverde CS V, Müller B, Rudnóy Sz, Fodor F (2021) Cadmium stress in Szarvasi-1 energy grass and its priming with S-methylmethionine. In: Összefoglaló kötet, XIII. Magyar Növénybiológiai Kongresszus, p7. (poster pres. Rana D)

Kolberg F, Tóth B, Rana D, Arcoverde Cerveira Sterner V, Gerényi A, Solti Á, Szalóki I, Sipos Gy, Fodor F. (2022) Iron supply determines zinc accumulation in Szarvasi-1 energy grass. In: Thomine, Sébastien Abtract Book of the 20th ISINIP, p. 121. (poster pres. Fodor F)

Müller B, Arcoverde Cerveira Sterner V, Papp L, May Z, Orlóci L, Sági L, Solti Á, Fodor F. (2023) Alkaline salt tolerance of the biomass plant Arundo donax A Magyar Szabadgyökkutató Társaság XII. Kongresszusa, Martonvásár ISBN 978-615-6203-02-1, p.47. (oral pres. Müller B)

Fodor F, Müller B, Solti Á, Arcoverde Cerveira Sterner V, Rana D, May Z, Sipos G. (2023) Increasing Tolerance of the Biomass Plant Szarvasi-1 Energy Grass to Abiotic Stresses by S-methylmethionine and Na-salicylate. In: Lekkas, Demetris F. (ed.) Book of Abstracts, 18th International Conference on Environmental Science and Technology, Athens, Greece, ISBN 978-618-86292-1-9, p.177. (oral pres. Fodor F)

Rana D, Arcoverde Cerveira Sterner V, Kumar Potluri A, May Z, Lantos B, Solti Á, Rudnóy Sz, Sipos Gy, Fodor F. (2023) Impact of Cd and Cu stress on physiological parameters and element accumulation pattern of Szarvasi-1 energy grass plants, A Magyar Szabadgyökkutató Társaság XII. Kongresszusa, Martonvásár, ISBN 978-615-6203-02-1, p.20. (poster pres. Rana D)

Arcoverde Cerveira Sterner V, Kristóf J, Rana D, Toth B, Sipos Gy , Fodor F. (2023) Salinity tolerance profiling of Szarvasi-1 energy grass at physiological, ionomical and gene expression levels. A Magyar Szabadgyökkutató Társaság XII. Kongresszusa, Martonvásár ISBN 978-615-6203-02-1, p. 68. (poster pres. Arcoverde CSV)

Arcoverde Cerveira Sterner V, Rana D, Sipos Gy, Fodor F. (2024) Towards the Establishment of a Technology Combining Phytoremediation and Biomass Production by Szarvasi-1 Energy Grass. In: Conference program, 6th Euro-Mediterranean Conference for Environmental Intergation, Marrakesh, Morocco. Paper 322. (oral pres. Fodor F)

Rana D, Arcoverde Cerveira Sterner V, Kumar Potluri A, May Z, Müller B, Solti Á, Sipos G, Fodor F (2024) Priming with S-methylmethionine reduces Cd effect and accumulation in Szarvasi-1 energy grass plants by increasing Fe uptake. In: Abstract Book of the 21th International Symposium on Iron Nutrition and Interactions in Plants, Düsseldorf, Germany. P33. (poster pres Fodor F)

Rana D, Arcoverde Cerveira Sterner V, May Z, Fodor F (2024) Heavy metal tolerance and ionomic composition of Szarvasi-1 energy grass. In: Összefoglaló kötet, XIV. Magyar Növénybiológiai Kongresszus, Szeged, Hungary. p36 (oral pres. Rana D)

Peer-reviewed publications

Müller B, Arcoverde Cerveira Sterner V, Papp L, May Z, Orlóci L, Gyuricza Cs, Sági L, Solti Á, Fodor F (2022) Alkaline salt tolerance of the biomass plant Arundo donax. Agronomy, 12(7): 1589. (IF=3.7, Q1)

Rana D, Arcoverde Cerveira Sterner V, Potluri AK, May Z, Müller B, Solti Á, Rudnóy Sz, Sipos Gy, Gyuricza Gy, Fodor F (2022) S-Methylmethionine effectively alleviates stress in Szarvasi-1 energy grass by reducing root-to-shoot cadmium translocation. Plants 11: 2979. (IF=4.5, Q1)

Kolberg F, Tóth B, Rana D, Arcoverde Cerveira Sterner V, Gerényi A, Solti Á, Szalóki I, Sipos G, Fodor F (2022) Iron Status Affects the Zinc Accumulation in the Biomass Plant Szarvasi-1. Plants 11: 3227. IF=4,5, Q1)

Arcoverde Cerveira Sterner V, Jobbágy K, Tóth B, Rudnóy Sz, Sipos Gy, Fodor F. (2024) Increasing salinity sequentially induces salt tolerance responses in Szarvasi-1 energy grass. Plant Stress 14: 100572. (IF_{2023}=6,8, D1)

Rana D, Arcoverde Cerveira Sterner V, May Z, Sipos Gy, Fodor F (2025) Combined heavy metal treatments cause marked changes in the ionomic patterns in Szarvasi-1 energy grass shoot biomass. Plant Soil https://doi.org/10.1007/s11104-025-07296-8 (IF=$3,9_{2023}$, D1)

Manuscript

Müller B, Arcoverde C.S. V, Pál M, Parádi I, May Z, Sipos Gy, Solti Á, Fodor F. (2025) Na-Salicylate and S-methylmethionine modulate stress-responsive ionomic and metabolomic pathways in Szarvasi-1 energy grass. *manuscript*