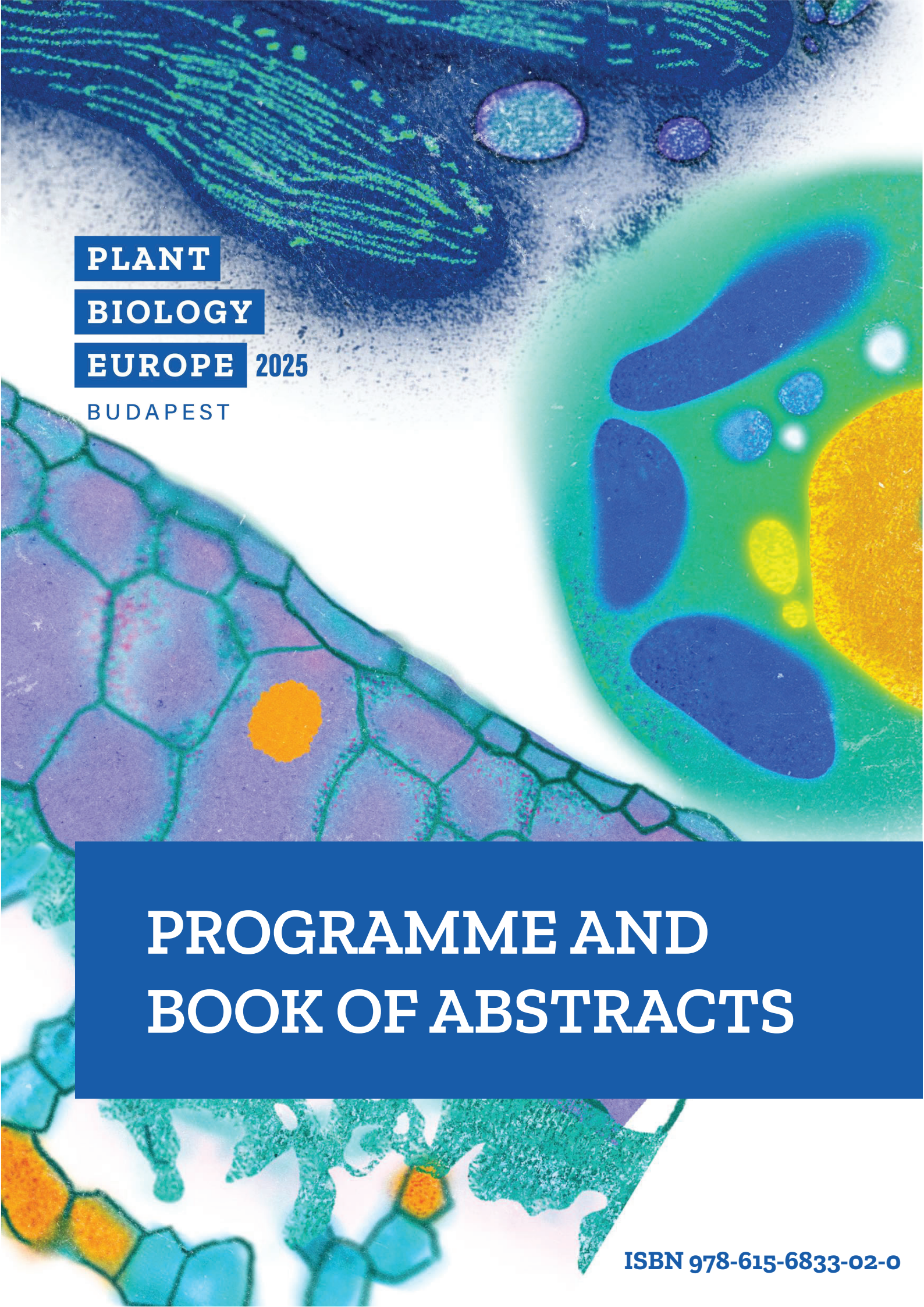

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ORAL PRESENTATIONS

We identified 72, 1290, and 2770 differentially expressed lncRNAs (DE-lncRNAs) during day-4, day-7, and day-10, respectively, in DILS, and four significant DE-lncRNAs in DLS. The target genes for these DE-lncRNAs were predicted (cis- and trans-manner), and the potential regulatory network of lncRNAs and their target genes was constructed. The target genes were involved in critical processes such as response to stress, cell redox homeostasis, transmembrane transport, protein folding and nitrogen compound transport. In this study, we report the first identification and characterisation of leaf senescence-associated lncRNAs in the barley genome, offering important insights for both barley and broader crop research. Our results are valuable for gaining deeper insights into the molecular mechanisms underlying leaf senescence, a complex and tightly regulated developmental process with important implications for crop improvement.

This work was supported by the National Science Centre, Poland, under the grant no. 2018/30/E/NZ9/00827to ES-N.

O-29

Building the edit suite: Foundational steps in hemp CRISPR technology

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Keywords: hemp, transformation, CRISPR

Hemp *(Cannabis sativa)* is a vital global crop, renowned for its adaptability, versatility, and rich history. However, unlocking the plant's full genetic potential for targeted breeding and sustainable agriculture requires overcoming the inherent challenges of its heterozygous nature and limited application of genetic techniques. While CRISPR has transformed gene editing in traditional crops, its application in hemp remains underdeveloped. This research aims to bridge this gap by establishing CRISPR-based genome editing techniques suitable for hemp, with the goal of advancing both basic research and applied breeding programs. Our approach involves developing a streamlined transformation process for stable gene editing in hemp. We use transformation methods *Agrobacterium*- based targeting hypocotyl explants in combination with RUBY, a robust reporter system for visual confirmation of successful transformations. Following transformation, rapid regeneration of both shoots and roots was observed at the hypocotyl cut sites within three-four days. Expression of the RUBY reporter was visually confirmed in the regenerated plant tissue. Initial observations suggest a mosaic expression pattern, with signal intensity increasing as the plant develops. These regenerated plants are currently undergoing incubation and will be subsequently transferred to soil for maturation and seed production. This work represents a crucial step towards establishing reliable genetic transformation protocols in hemp, enabling efficient CRISPR/Cas-mediated genome editing and accelerating targeted trait improvement research.

O-30

Validation of the DRO1 gene in tomato under drought stress: Insights into root architecture and drought adaptation

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Keywords: root architecture, drought, CRISPR-Cas9

Drought stress severely limits crop productivity, highlighting the need for strategies to improve water use efficiency while maintaining yield stability. Optimizing root architecture, particularly through genes like *Deeper Rooting 1 (DRO1)*, offers a climate-resilient approach to enhance drought adaptation. *DRO1* has been identified as a key quantitative trait locus (QTL) in rice and Arabidopsis, promoting deeper root growth and access to groundwater during drought. However, root traits like angle and length are species-specific, underscoring the need to explore this gene's role in other crops. This work aims to functionally characterize *DRO1*-mediated root architecture in tomato (*Solanum lycopersicum L.*), a globally important crop. Using an omics-driven approach combining phenomics and genomics, the project investigates the spatio-temporal regulation of *DRO1* and its role in shaping root architecture under drought in the Micro-Tom cultivar.

Root system analysis, including scanning and WinRHIZO, revealed a decrease in root length and very fine root fraction after 30 days of drought, while the root mass ratio increased, indicating a shift in resource allocation.

Imaging techniques showed stable root surface area and fineness across time points. Near-infrared (NIR) imaging on a LemnaTec 3D Scanalyzer confirmed reduced water content in epigeal part of drought-stressed plants, as indicated by increased reflectance. These findings highlight key root functional traits and spectral indices for assessing drought adaptation.

qPCR analysis showed that *SlDRO1* expression in roots remained stable at early stages but increased significantly after 20 days of drought. In contrast, expression in stems exhibited the opposite trend, with higher values limited to the pre-stress stage. This suggests tissue-specific regulation of *SlDRO1*, with drought-induced activation in roots, potentially supporting its role in adaptive responses to water scarcity. In situ hybridization studies further localized *SlDRO1* expression in both stems and roots, providing spatial insights into its role during drought adaptation.

Ongoing efforts are focused on generating knockout (KO) and overexpression (OEX) lines of *SlDRO1* to enable full functional characterization of the gene in tomato, a first for this species. These findings will advance our understanding of the genetic determinants underlying root system plasticity and contribute to improving drought resilience in tomato and other crops.

This work was supported by the European Union - Next-GenerationEU - National Recovery and Resilience Plan – MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, CALL PRIN 2022 PNRR

O-31

Identification of quantitative trait loci (QTL) underlying protein content in chickpea (*Cicer arietinum* L.) seeds

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Keywords: chickpea, seed protein content, quantitative trait loci (QTL), linkage analysis, genome-wide association study (GWAS); candidate genes

Chickpea (*Cicer arietinum L.*) is the second most important legume crop for human consumption. Being a legume, chickpea plays a crucial role in increasing agriculture sustainability by improving food security and nutrition and facilitating the transition towards plant-based diets. Indeed, its high seed protein content (17%-22%) makes the species very attractive to be used as protein source alternative to meat.

Here we characterized a wide panel of chickpea domesticated genetic resources representative of the entire geographic distribution of the species, genetically and for seed protein content, with the aim to identify genomic regions associated with protein content using a genome-wide association study (GWAS) approach.

We cultivated 202 Single Seed Descent (SSD) chickpea lines in two different field trial carried out in central Italy in two years. Seeds were analyzed for crude protein content and Whole Genome Sequencing (WGS) data were used to perform GWA.

Preliminary analyses revealed eight significant marker-trait associations (MTAs) related to protein content, distributed across chromosomes 2, 5, and 6.

These findings represent a useful tool for breeding aimed at developing chickpea varieties characterized by high nutritional value.

O-32

Utilization of targeted mutations to alter economically important traits in barley

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Keywords: barley, genome editing, traits

Genome editing is a revolutionary technology allowing the targeted introduction of genetic changes into the genome. We try to utilize this technology to alter various traits in barley which can potentially be useful in future crop improvements.

Width and Weight 2 (GW2) is an E3-ubiquitin ligase-encoding gene that negatively regulates the size and weight of the grain in cereal species. Therefore, disabling GW2 gene activity was suggested for enhancing crop

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