COVER

Name of the Center:	
Type (Institute or Nucleus)	Institute
Acronym	iBio
Reported period	January 1st to December 31, 2024
Starting date of the Center	December 26Th 2017
Web Page	www.ibio.cl
Host Institution(s)	UC, USACH, UMayor, UNAB
Address	General del Canto 50, of 301, Providencia
Stage	Continuity
Year of Execution	2024
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Total amount for the reported period	USD $895.522

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Singapore Declaration
I hereby declare that the information provided in this Annual report is reliable, and that I adhere to the Singapore Declaration, contained in Conicyt Exempt Resolution No. 157 of January 24, 2013, as a global guide for responsible conduct in research.

Institute / Nucleus Director Name	*Institute / Nucleus Alternate Director Name*
Luis Larrondo C.	*Rodrigo Gutierrez I.*
Director's Signature	*Alternate Director's Signature*

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1. Introduction & Executive Summary

The iBio Institute brings together an outstanding team of researchers working synergistically to uncover how plants and fungi respond to environmental (abiotic) and biological (biotic) signals, and how these interactions unfold across space and time in a changing world. Our research explores how nutrients, light, and temperature cues, as well as inter-organismal interactions shape organismal molecular and physiological responses, gaining mechanistic insights on fundamental aspects of plant and fungal biology, but also with the potential to be applied in the productive sector, particularly fostered by innovative synthetic biology and biotechnology strategies.

Our work is structured around four highly integrated and collaborative research lines (RLs).

RL1 and RL2 focus on understanding how organisms perceive and respond to environmental signals. RL3 builds on this knowledge to explore how organismal interactions can be understood and manipulated across spatial and temporal scales. RL4, in turn, develops new enabling technologies that support and amplify the work across the other research lines. Beyond advancing fundamental knowledge in plant and fungal sciences, our research is contributing to emerging biotechnological innovations with real-world applications, particularly in the food industry.

The iBio core scientific team has remained cohesive and stable over the years. It is led by Director Luis Larrondo (LL), Deputy Director Rodrigo Gutiérrez (RG), and associate researchers Elena Vidal (EV), Francisco Cubillos (FC), Paulo Canessa (PC), and Fernán Federici (FF)—all of whom are engaged in pioneering research in fungal and plant biology and genetic circuits. The team is further supported by eight adjunct researchers: Francisca Blanco (FB), Mariana Gómez-Schiavon (MGS), Francisco Salinas (FS), Roberto Nespolo (RN), Javier Canales (JC), César Ramírez-Sarmiento (CRS), José Estevez (JE), and José Miguel Álvarez (JMA).

In addition to its principal investigators, iBio supports the development of future leaders in science through its "Young Investigators" program, which includes senior postdoctoral researchers who are launching their independent careers. The institute also plays a key role in training the next generation of scientists. In 2024, a total of 46 undergraduate students, 46 graduate students, 21 postdoctoral researchers, and 12 Young Investigators conducted research in iBio-affiliated labs. Among them, 10 undergraduate students and 6 graduate students (2 Ph.D. and 4 Master's) successfully completed their academic programs. The success of our students and professionals is evident, as many have secured positions aligned with their research interests in academia, the private sector (industry), and the public sector.

During 2024, iBio forged significant national and international collaborations. Notably, we continue our partnership with the National Center for Artificial Intelligence to co-develop a large language model (LLM) specialized in biology and designed to improve access to academic literature in the life sciences. Our partnership with Fundación Ciencia y Vida (FCV) has further expanded our technological, scientific, and technology transfer capabilities. Internationally, our collaboration with the RIKEN Center for Sustainable Resource Science is enabling us to advance research on drought and heat stress, with the aim of improving the resilience and yield of perennial crops—an urgent priority for the future of global agriculture. Likewise, our new partnership with Standford Bio-X will potentiate our efforts in exploring new fungal-based food alternatives.

Our main outreach initiative this year was aligned with a core mission of our center: promoting the use of Open Science tools in education and research. The project focused on the development and dissemination of open-source scientific instruments, free software, and locally produced biotechnological reagents. Its primary goal was to train high school teachers in the use of Open Educational Resources (OER) that integrate open hardware, free software, and accessible biotechnological tools to enable hands-on instruction in molecular biology. Launched in December 2024, this initiative has already delivered strong results, with more than 30 high school biology teachers trained in southern regions of Chile—areas where access to this type of educational resource is often limited or nonexistent.

iBio has continued to strengthen its presence on the national stage, frequently appearing on television, radio, and in print media. In 2024, we reached a new milestone with over 223 media appearances. This number reflects iBio's longstanding commitment to scientific communication, demonstrated over the years through publications across various media platforms and social networks using an accessible, engaging, and dynamic language.

In summary, 2024 was a year of significant scientific progress, strategic collaborations, and meaningful communication and societal impact for iBio. By combining fundamental research with technological innovation and outreach, we continue to build a platform that not only advances scientific understanding but also strengthens the national research ecosystem and fosters the next generation of scientific leaders.

1.1 Resumen Ejecutivo

El Instituto iBio reúne a un destacado equipo de investigadoras/es que trabajan de manera sinérgica para descubrir cómo plantas y hongos responden a señales ambientales (abióticas) y biológicas (bióticas), y cómo estas interacciones se desarrollan en el espacio y el tiempo en un mundo cambiante. Nuestra investigación explora cómo las señales de nutrientes, luz y temperatura, así como las interacciones entre organismos, moldean respuestas moleculares y fisiológicas, generando conocimiento mecanístico sobre aspectos fundamentales de la biología vegetal y fúngica, con un alto potencial de aplicación en el sector productivo, especialmente a través de estrategias innovadoras de biotecnología y biología sintética.

Nuestro trabajo se estructura en torno a cuatro Líneas de Investigación (LI) altamente integradas y colaborativas.
LI1 y **LI2** se centran en comprender cómo los organismos perciben y responden a señales ambientales.
LI3 se basa en este conocimiento para explorar cómo se pueden comprender y manipular las interacciones entre organismos a distintas escalas espaciales y temporales.
LI4, por su parte, desarrolla nuevas tecnologías habilitantes que respaldan y amplifican el trabajo de las otras líneas de investigación.

Más allá de avanzar en el conocimiento fundamental en ciencias vegetales y fúngicas, nuestra investigación está contribuyendo a innovaciones biotecnológicas emergentes con aplicaciones concretas, especialmente en la industria alimentaria.

El equipo científico principal de iBio se ha mantenido cohesionado y estable a lo largo del tiempo. Está liderado por el Director Luis Larrondo (LL), el Director Alterno Rodrigo Gutiérrez (RG), y los investigadores asociados Elena Vidal (EV), Francisco Cubillos (FC), Paulo Canessa (PC) y Fernán Federici (FF), todos ellos involucrados en investigaciones pioneras en biología fúngica, vegetal y circuitos genéticos.
Este equipo cuenta además con el apoyo de ocho investigadores adjuntos: Francisca Blanco (FB), Mariana Gómez-Schiavon (MGS), Francisco Salinas (FS), Roberto Nespolo (RN), Javier Canales (JC), César Ramírez-Sarmiento (CRS), José Estévez (JE) y José Miguel Álvarez (JMA).

Además de sus investigadores principales, iBio apoya el desarrollo de futuros líderes científicos a través de su programa de "Jóvenes Investigadores", que incluye a investigadores postdoctorales senior que están iniciando sus carreras independientes. El instituto también cumple un rol clave en la formación de la próxima generación de científicos. En 2024, un total de 46 estudiantes de pregrado, 46 estudiantes de posgrado, 21 investigadores postdoctorales y 12 Jóvenes Investigadores realizaron investigación en laboratorios asociados a iBio. De ellos, 10 estudiantes de pregrado y 6 estudiantes de posgrado (2 doctorados y 4 magíster) finalizaron exitosamente sus programas académicos. El éxito de nuestros estudiantes y profesionales es evidente, ya que muchos han logrado insertarse en posiciones acordes a sus intereses científicos en el ámbito académico, industrial y público.

Durante 2024, iBio estableció colaboraciones significativas a nivel nacional e internacional. Destaca la continuidad de nuestra alianza con el Centro Nacional de Inteligencia Artificial para el desarrollo conjunto de un modelo de lenguaje de gran escala (LLM) especializado en biología, diseñado para mejorar el acceso a literatura académica en ciencias de la vida. Nuestra colaboración con la Fundación Ciencia & Vida (FCV) ha fortalecido aún más nuestras capacidades tecnológicas,

científicas y de transferencia.

A nivel internacional, nuestra colaboración con el RIKEN Center for Sustainable Resource Science nos ha permitido avanzar en investigaciones sobre estrés por sequía y calor, con el objetivo de mejorar la resiliencia y productividad de cultivos perennes —una prioridad urgente para el futuro de la agricultura global. Asimismo, nuestra nueva alianza con Stanford Bio-X potenciará nuestros esfuerzos en el desarrollo de nuevas alternativas alimentarias basadas en hongos.

Nuestra principal iniciativa de vinculación con el medio durante este año estuvo alineada con una misión fundamental del centro: promover el uso de herramientas de Ciencia Abierta en la educación e investigación. El proyecto se enfocó en el desarrollo y la difusión de instrumentos científicos de código abierto, software libre y reactivos biotecnológicos de producción local. Su objetivo principal fue capacitar a docentes de enseñanza media en el uso de Recursos Educativos Abiertos (REA) que integran hardware libre, software libre y herramientas biotecnológicas accesibles, para permitir una enseñanza práctica en biología molecular.

Lanzada en diciembre de 2024, esta iniciativa ya ha mostrado resultados concretos, con más de 30 profesores de biología de enseñanza media capacitados en regiones del sur de Chile, donde el acceso a este tipo de recursos educativos suele ser limitado o inexistente.

iBio ha seguido fortaleciendo su presencia a nivel nacional, apareciendo con frecuencia en televisión, radio y prensa escrita. En 2024, alcanzamos un nuevo hito con más de 223 apariciones en medios, lo que refleja el compromiso sostenido de iBio con la comunicación científica, demostrado a lo largo de los años mediante publicaciones en diversos medios y redes sociales, utilizando un lenguaje accesible, atractivo y dinámico.

En resumen, 2024 fue un año de importantes avances científicos, colaboraciones estratégicas y un impacto significativo en la comunicación y la sociedad para iBio. Al combinar investigación fundamental, innovación tecnológica y vinculación con la sociedad, seguimos construyendo una plataforma que no solo impulsa el conocimiento científico, sino que también fortalece el ecosistema nacional de investigación y forma a la próxima generación de líderes científicos.

1.2. Outstanding Achievements

As we review the performance of our Institute, it is impossible not to highlight the academic and personal quality of our community members: young students and researchers, who have grown both professionally and personally alongside the Center, allowing our institute to also grow and evolve. It is a source of great pride and satisfaction to see them securing their own sources of funding, enabling them to develop independent research lines that will, in turn, create new opportunities for researchers and students.

As we explain in the next section, we achieved significant scientific advances in 2024. For example, in plants we uncovered new mechanisms by which nitrate regulates cotyledon growth, modulating cytokinin signaling, endoreplication, and cell wall metabolism. We also constructed organ-specific gene networks in tomatoes and identified regulatory elements driving stress resilience through *cis*-regulatory evolution. In fungi, we introduced the concept of "conditional clocks" and revealed evolutionarily conserved circadian mechanisms. We further documented the plant growth-promoting effects of diverse fungal isolates, and also put forward the idea of "optoecology," expanding the frontier of optogenetic applications.

Among the 41 papers published this year, highlights include the description of *Saccharomyces chiloensis*, a novel *Saccharomyces* species that underscores the microbial biodiversity of Chilean ecosystems, and the development of a semi-synthetic circadian clock that deepens our understanding of how organisms perceive environmental cues. We also elucidated mechanisms underpinning fungal-fungal interactions, with implications for biocontrol. Using environmental metabolomics, we provided insights into the mechanisms for wild species facilitation under harsh conditions. We also showed the influence of plant metabolism on the chemical rhizosphere and associated microbiome.

Our students received awards at national and international conferences, reflecting both the scientific excellence and communication skills fostered in our center. Additionally, the director and deputy directors were honored with major recognitions, being elected to the Chilean Academy of Sciences and EMBO, respectively.

During this period, we focused our efforts on building collaborations with renowned national and international institutions, aiming to continually enhance the quality of our research and acquire the knowledge and technological capabilities needed to generate a positive local impact—both in the region and within our communities.

We are proud to bring science to remote corners of our country, supporting the education of teachers and students at the school level, with the goal of promoting equitable and direct access to opportunities for young people, from the classroom to their professional development.

2. Scientific and technological research:

2.1 Methodological and technical procedures:

Scientific Question: The unifying question at the heart of iBio's mission is: "How do environmental signals (both abiotic and biotic) interact with gene regulatory networks in plants and fungi to control their development, physiology, and inter-organismal interactions, and how can this knowledge be harnessed through synthetic and systems biology strategies to create sustainable, open-source biotechnological solutions for agriculture and food systems?

Our scientific activity can be summarized into 4 main research lines (see below). **RL1** and **RL2** address key aspects of how organisms sense and respond to the environment. **RL3** uses this information to manipulate/understand organismal interactions in space and time. In turn, **RL4** focuses on generating and enabling new technologies that we can apply to **RL1**, **RL2** or **RL3**.

Research Line 1 (**RL1**): Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.
Research Line 2 (**RL2**): To Assess the Effect of Environmental Signals on Interspecies Dynamics.
Research Line 3 (**RL3**): Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.
Research Line 4 (**RL4**): Implementation of open source technologies and promotion of open science.

Thus, we seek to unravel the genetic and cellular mechanisms involved in fungal and plant responses to environmental signals such as light, temperature and nutrient availability with temporal and spatial resolution (**RL1**). Organismal growth and development, as well as their ability to respond adequately to environmental changes/stresses are significantly influenced by interactions with other organisms in their local environment. Unraveling mechanisms that explain these organismal interactions, and how they are modulated by abiotic and temporal cues is a central task (**RL2**). On the other hand, adopting new species, beyond classic models, can provide a better perspective on niche specific traits, or general principles, and is something we have started to do. In addition, multi-approach strategies in the implementation of transcriptional switches for the control of diverse processes and for the rewiring of metabolic functions can lead to the advancement of applied technologies and solutions, while also allowing the study of complex processes such as transcriptional memory (**RL3**). Finally, active programs in Open Source Technologies and do-it-yourself approaches for more accessible science are exploited as a powerful platform to power low cost/high-impact research and to connect science with society (**RL4**)

Methodological Approach: From a methodological standpoint, we employ a broad range of cutting-edge technologies, alongside classical approaches, to investigate both the proximal and ultimate causes underlying phenomena related to how organisms sense and respond to environmental fluctuations. As will become clear in the next section, our research integrates systems biology, comparative genomics, and synthetic biology to dissect gene regulatory networks (GRNs) and uncover novel biological principles in both plants and fungi. We reconstruct GRNs using co-expression analyses, motif enrichment, and transcription factor (TF)–target predictions, guided by high-resolution datasets including bulk RNA-seq, ploidy-resolved transcriptomics, and single-nucleus RNA-seq. These efforts are complemented by synthetic biology strategies -such as transcriptional rewiring and optogenetic switches- to experimentally perturbe and reprogram regulatory circuits *in*

vivo. We also employ whole-genome sequencing and integrative taxonomy to identify novel species and explore functional diversity among wild isolates. Additionally, our studies use mutational dissection, NMR-guided biochemical assays, and phosphoproteomics to reveal key regulatory mechanisms. This systems-level approach is complemented by quantitative phenotyping platforms (e.g., WS-YOLO, LightCycler) and classical genetics, enabling a mechanistic bridge from molecular circuits to organismal behavior. Through these integrative strategies, we seek to uncover principles of regulation that not only advance basic science but also hold translational value for sustainable biotechnological applications.

Other Aspects: The results obtained over the past year are in line with our expectations and our overall long-term plan. As outlined in the following section, we devoted significant efforts to promoting open technologies and expanding access to reagents. Whenever possible—and considering the lack of open-access (OA) discount agreements between major publishing companies and Chilean institutions—we have published our work in OA venues.

Difficulties: Fortunately, we have not encountered major setbacks or issues affecting our scientific progress or day-to-day activities.

2.2 Associative & collaborative work:

The iBio core scientific team has remained cohesive and stable throughout these 7 years, and is composed of the institute Director (Luis Larrondo, ***LL***), Deputy Director (Rodrigo Gutiérrez, ***RG***) and four **associate researchers** (Elena Vidal, ***EV***; Francisco Cubillos, ***FC***; Paulo Canessa, ***PC***; Fernán Federici, ***FF***), all of them participating in pioneer research programs in fungal, plant biology and genetic circuits. The iBio scientific team also includes 8 **adjunct researchers**: Francisca Blanco, ***FB***; Mariana Gómez-Schiavon, ***MGS***; Francisco Salinas, ***FS***; Roberto Nespolo, ***RN***; Javier Canales, ***JC***; César Ramírez-Sarmiento, ***CRS***; José Estevez, ***JE*** and José Miguel Álvarez ***JMA***. In iBio we also have the category of "**Young Investigators**", which corresponds to senior postdocs that are starting their independent career, obtaining independent funding, achieving academic positions, or applying to them as they share space and work with main iBio PIs.

Our work continues to reveal clear areas of overlapping interest, leading to meaningful collaborative efforts among both associate and adjunct researchers. In the following section, we highlight how diverse research areas actively involve multiple PIs. While such collaborations are evident in our published work, it is in our ongoing unpublished projects where these associative efforts are most reflected and fill us with enthusiasm. Importantly, students -both undergraduate and graduate- along with postdocs and early-career investigators, play a key role in bridging collaborative efforts across labs. We have multiple examples of co-tutored undergraduate and graduate students, shared postdoctoral researchers, and young investigators facilitating intra and inter-lab initiatives.

Due to space constraints, the next section provides only a concise overview of selected research projects, although many additional ongoing projects further exemplify the collaborative spirit that defines iBio. Just to provide a couple of examples, studies on different plant growth–promoting fungi involve collaborations between *i)* ***EV***, ***LL***, and ***FC***; *ii)* ***RG*** and ***LL***; and *iii)* ***FC***, ***PC***, ***LL***, and ***JE***. Work on gene regulatory networks (GRNs) and stress involves ***EV*** and ***JC***, as well as ***JMA*** and ***EV***. Projects dissecting GRNs related to plant cell wall deconstruction involve ***LL*** and ***RG***, investigations into fungal nitrogen homeostasis and clock regulation bring together ***LL***, ***PC***, and ***RG***, while optogenetics and Open technologies efforts involve collaborations between ***FF***, ***LL***, ***FC***, ***FS*** and ***PC***.

2.3 Results and impact: (*Please find accompanying Figures in Annex 9*)

RL1. Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.

Nitrate and cytokinin interaction in the modulation of cotyledon growth (*RG*): Nitrate, a key nitrogen source and signaling molecule, plays a crucial role in promoting leaf growth by influencing the cell cycle. It stimulates both cell proliferation in leaf primordia and endoreplication in cotyledons. The latter, which is a cell cycle variation in which DNA synthesis occurs without cell division, leads to increased ploidy and cell size. Given cytokinin's (CK) known role in regulating shoot development and the cell cycle, we investigated whether nitrate modulates cotyledon growth through CK-dependent mechanisms in *Arabidopsis thaliana*. Using flow cytometry, confocal microscopy, and genetic tools—including mutant lines and fluorescent reporters—we analyzed cotyledon size, epidermal cell area, ploidy levels, and active CK signaling under different nitrate and CK conditions. Our results show that CK restricts cotyledon growth by limiting cell expansion and delaying the onset of endoreplication under sustained nitrate conditions. In addition, we found nitrate accelerates and downregulates active CK signaling in the cotyledon epidermis. Our data support a working model where nitrate regulates active CK signaling during cotyledon development, modulating the transition from cell cycle to endoreplication and ultimately cotyledon growth (**Fig. 1**).

Nitrate, Ploidy, and Cell Identity (*RG*, *EV*): To understand how nitrogen availability regulates plant growth at the cellular level, we combined single-nucleus RNA sequencing (snRNA-seq) and ploidy-resolved transcriptomics in *A. thaliana*. Using snRNA-seq on cotyledons grown under three nitrate conditions (0, 0.5, and 5 mM), we obtained 4,558 high-quality single-nucleus transcriptomes across 21 cell-type clusters. We identified major cell types, including epidermal, mesophyll, vascular, and dividing cells, and are currently performing pseudo bulk differential expression and gene regulatory network analysis to uncover nitrate-responsive transcriptional regulators. In parallel, we investigated the impact of nitrate-induced endoreplication by sequencing nuclei sorted by ploidy (2C to 16C). We identified over 5,000 differentially expressed genes, with subsets responsive to nitrate, ploidy, or both. Genes upregulated under high nitrate were enriched in metabolism and cell cycle processes, while those linked to low nitrate reflected stress responses. GO analysis of ploidy-specific expression clusters revealed distinct developmental and differentiation states (**Fig. 2)**. These findings highlight nitrate's role in coordinating gene expression, cell fate, and expansion through both transcriptional and endoreplication-dependent mechanisms.

Unraveling the interplay between nitrate and cell wall metabolism (*RG*). Integrative transcriptome analyses revealed that nitrate availability influences cell wall organization, where plants grown under high nitrate exhibited altered cellulose and pectin (previous report). We observed that nitrate-enhanced growth was associated with increased CESA velocity and nitrate-dependent phosphorylation of the CESA3 subunit. Now, we present evidence that key components of the nitrate signaling pathway, including NRT1.1 and CIPKs 10, 30, and 32, mediate CESA3 phosphorylation, thereby regulating cellulose synthesis and accumulation in response to nitrate. Regulation of CESA3 phosphorylation by nitrate signaling is critical for CESA complex function and cellulose deposition to enable growth (**Fig. 3**). Our results highlight changes in cellulose synthesis and also pectin metabolism, as part of the regulation of shoot growth to ensure cell wall changes required for cell expansion in response to nitrate.

Organ-Specific Gene Networks Reveal Regulatory Complexity in Tomato (*EV*, *JC*): *Solanum lycopersicum* (tomato) is a vital crop, yet its gene regulatory networks (GRNs) remain poorly understood. To bridge this gap, we constructed organ-specific GRNs for roots, leaves, flowers, fruits, and seeds by analyzing over 10,000 RNA-seq libraries using the GENIE3 algorithm. The networks incorporated co-expression data and TF binding predictions. The GRNs successfully recapitulated experimentally validated targets for the TFs, highlighting their reliability. As proof of concept, fruit

and leaf GRNs were created for key regulators: TAGL1 and RIN (ripening), and ABF3/ABF5 (ABA response). These GRNs recapitulated known TF targets based on ChIP-seq and RNA-seq validation (**Fig. 4 and 5**). Novel candidate regulators, such as SlARF2A, SlERF.E2, and SlGBF3, were identified. To promote accessibility, the TomViz web platform was developed following FAIR principles, allowing interactive exploration of tomato GRNs and supporting future crop improvement research. TomViz is implemented as an RShiny-based application integrated into the PlantaeViz platform (Santiago et al., 2024 doi.org/10.1101/2024.12.19.629382.).

Stress resilience (*JMA*, *EV*): Understanding how plants adapt to environmental stress is crucial, especially under increasing drought conditions due to climate change in subtropical regions. Traditionally, most studies focus on sensitive model species, limiting our grasp of naturally evolved resilience. Yet, extremophytes, like the wild tomato *Solanum pennellii*, can be readily compared to drought-sensitive crops such as *S. lycopersicum*. Using comparative transcriptomics and promoter analyses under drought, we found that ~43% of drought-responsive orthologs showed species-specific expression, with ~20% displaying a "stress-ready" profile in in *S. pennelli* (Contreras-Riquelme et al., PMID: 40392843). Promoter motif analysis confirms significant enrichment of ERF transcription factor motifs in the *S. pennellii* drought-responsive genes, suggesting that *cis*-regulatory evolution shapes species-specific GRNs underlying relevant traits such as stress resilience (**Fig. 6)**.

Heat Stress Responses in Wheat (*JC*, *EV*): We investigated the molecular responses of wheat species with different ploidy levels—*Triticum monococcum* (diploid), *T. turgidum* (tetraploid), and *T. aestivum* (hexaploid)—to acute heat stress during early grain filling. We applied controlled thermal treatments to field-grown plants and performed transcriptomic and alternative splicing (AS) analyses after three hours of exposure. Our comparative transcriptome analysis revealed that *T. aestivum* exhibited the most extensive transcriptional reprogramming (2,227 DEGs), followed by *T. monococcum* and *T. turgidum*. While all species activated core heat stress pathways, such as protein folding and oxidative stress responses, *T. aestivum* uniquely enriched pathways related to mRNA processing and spliceosome function, suggesting a role for alternative splicing (AS) in heat adaptation. AS analysis showed that *T. aestivum* had the highest number of heat-induced splicing events, including exon skipping in the NFY-B transcription factor. RT-PCR confirmed temperature-dependent isoform switching (**Fig. 7**). Our results suggest that genome complexity enhances heat stress resilience by integrating transcriptional and co-transcriptional regulation, providing insights for breeding heat-tolerant wheat varieties.

Structure-Function of circadian components (*LL*): Circadian clocks allow precise coordination of organismal biology with environmental cycles. These clocks rely, from fungi to humans, on tightly regulated kinase activity to ensure precise timing of physiological processes. Casein Kinase 1a (CK1) plays a central role in period determination, yet the contribution of its disordered C-terminal tail has remained largely unexplored. In the circadian model fungus *Neurospora crassa* we identified a 23-residue segment encoded by the gene's exon 3 of *ck1*, termed the E3 region, as a critical determinant of circadian timing, detecting that this positively charged region modulates period length through a phosphorylation-sensitive electrostatic mechanism. Genetic dissection of CK1 isoforms reveals that the presence of E3 extends circadian period, while its absence, or its neutralization through phosphomimetic mutations, accelerates the clock, whereas addition of positive charges strongly delays it (**Fig. 8**). NMR studies show that E3 charge-characteristics modulates CK1a's phosphorylation efficiency toward specific circadian substrates, such as the FRQ PEST1 domain (**Fig. 9**). Notably, the human tail, when fused to a short version of *N. crassa* CK1 complements function in Neurospora. Taken together, these findings uncover a deeply conserved biochemical strategy in which

posttranslational electrostatic regulation of CK1 isoforms operates across evolutionary boundaries -from fungi to humans- highlighting a unifying principle in circadian period control (Costa et al., *in preparation*).

Biomass deconstruction (*LL*, *RG*): We previously identified 14 TFs involved in lignocellulose degradation based on a predicted *N. crassa* GRN we assembled. Functional analyses of these candidates on various lignocellulosic substrates revealed that five TFs -including NCU03043 and NCU06656- significantly impacted growth, protein secretion, and enzymatic activity. Genetic crosses indicated their involvement in at least two distinct regulatory pathways, while rewiring experiments demonstrated that NCU06656 functions upstream of *clr-2*, the primary TF known to regulate cellulolytic activity. Notably, deletion of NCU01871 led to protein over-secretion when grown in sucrose, and reduced secretion in starch, indicating a context-specific regulatory role. Collectively, these findings advance our understanding of PCWDE regulation and offer tools for engineering fungi for efficient biomass conversion (**Fig. 10**).
In addition, we have systematically started comparing the fungi *N. crassa* and *Neurospora intermedia*, in their ability to grow and process subproducts of the food industry (i.e. slush from soy or coconut-milk), in order to upcycle those industrial residues. Notably, *N. crassa* is as efficient as *N. intermedia* in these tasks, and we are currently analyzing how environmental factors (light and temperature) change the speed of the process, as well as the organoleptic properties of the fermented end-product.

New natural and evolved yeast species (*FC*, *LL*): Over the years, we have devoted significant efforts to identifying new yeast species and isolates in the Chilean Patagonia. Until recently, we had not succeeded in identifying a new *Saccharomyces* species, making this achievement a major milestone (Peña et al., PMID: 39241096). The discovery of *Saccharomyces chiloensis* sp. nov. (and the phenotypic diversity of the isolates) highlights the exceptional biodiversity of Patagonia's coastal ecosystems and emphasizes the value of integrative taxonomy in uncovering cryptic microbial species and refining species boundaries (**Fig. 11**). Furthermore, this work introduces an innovative approach to describing new yeast species through whole-genome sequencing, complementing our experience in species identification, such a yeast (*Starmerella guilliamae*) isolated from the flowers of *Neltuma chilensis* (Algarrobo), a key species in Chilean ecosystems (Rosa et al., PMID: 38407127).
We also focused on expanding the genetic and flavor diversity of lager yeasts by harnessing the natural variation of *Saccharomyces eubayanus* lineages from Patagonia. To this end, we generated *S. cerevisiae* × *S. eubayanus* hybrids and applied experimental evolution to enhance fermentation performance, ethanol production, and aroma complexity. Hybrids carrying *S. eubayanus* mitochondria exhibited superior fitness and evolutionary potential. Through genome-wide analyses, we identified mutations and gene copy variations in *IRA2*, *IMA1*, and *MALX* that improved maltose metabolism and glycolytic flux. Functional assays and transcriptomic data confirmed increased maltotriose consumption (Molinet et al., PMID: 38900713). This work represents a significant scientific advance toward developing novel lager strains from Patagonia and was highlighted by international media outlets such as *The Economist* and *The Washington Post*.
In addition, after observing large phenotypic variation in maltose consumption among wild *S. eubayanus* isolates, we developed an *in vivo* system to monitor transcriptional activation during the glucose-to-maltose metabolic shifts in *S. eubayanus* (**Fig. 12**). This allows us to assess, at the molecular level, the basis of these differences (Muñoz-Guzmán et al., PMID: 40063507).

(RL2) To Assess the Effect of Environmental Signals on Interspecies Dynamics.

***Botrytis cinerea* Response Against *Trichoderma atroviride* (*PC*).** *Botrytis cinerea* is a destructive necrotrophic fungus that infects over 1,000 plant species, posing a major threat to agriculture. While *Trichoderma atroviride* is an effective biocontrol agent against *B. cinerea*, the latter fungus defense mechanisms against mycoparasitism remain poorly understood. Through GRNs analyses we identified four *B. cinerea* transcription factors (TFs) involved in fungal-fungal defense, with BcMTF1 (Bcin07g06800) playing a key role. Δ*bcmtf1* mutants showed heightened susceptibility to *T. atroviride*, while overexpression of *bcmtf1* enhanced resistance (**Fig. 13)**. Notably, BcMTF1 had no effect on *B. cinerea*'s virulence in plant hosts, indicating a defense role specific to fungal antagonism (**Fig. 14**). RT-qPCR of predicted BcMTF1 target genes during the *B. cinerea–T. atroviride* interaction revealed three distinct expression patterns: BcMTF1-dependent upregulation, BcMTF1-dependent regulation (up and down), and BcMTF1-independent expression. Genes involved in stress response and protease production were highly dependent on BcMTF1 presence. These findings position BcMTF1 as a promising target to boost biocontrol efficacy without affecting pathogenicity, offering new strategies for sustainable crop protection (Olivares-Yañez et al., PMID: 39925430)

Integration of metabolic pathways and organismal interactions (*LL*, *PC*, *RG*): We have played a pioneering role in characterizing clock and light-sensing mechanisms in fungi with key ecological functions, whether as pathogens or biocontrol agents. In the course of this work, we uncovered unexpected interconnections between metabolic pathways and circadian mechanisms. Notably, we coined the concept of "conditional clocks" (Larrondo, PMID: 39842475) to describe circadian systems that remain functionally "dormant" under specific metabolic conditions, but become active when alternative nutritional cues are present. This phenotype is particularly pronounced in the biocontrol fungus *T. atroviride*, as well as in *Alternaria alternata*, where we are currently investigating the role of MAP kinase pathways and plant-derived signals in modulating this behavior.
In *B. cinerea*, the clock protein ortholog BcFRQ1 plays roles not only in circadian regulation but also in development and metabolism (Olivares-Yañez et al., in prep). Its deletion results in an "always-sclerotia" phenotype, which can be reversed by supplying primary nitrogen sources, thus linking BcFRQ1 to nitrogen metabolism. The Δ*bcfrq1* mutant also exhibits strong derepression of secondary metabolism (SM). Using TurboID-based proximity labeling combined with mass spectrometry under nitrate and glutamine conditions, we identified nitrogen-dependent BcFRQ1 interactors, including key enzymes such as nitrate and nitrite reductases (**Fig. 15**). Interestingly, the transcription factor NirA, but not AreA, showed enhanced interaction with BcFRQ1 under nitrate, suggesting a role in nitrogen signaling. Additionally, BcFRQ1 associates with SM regulators such as BcLAE1 and BcVEL2 of the VELVET complex, indicating that clock components contribute to metabolic and developmental regulation beyond their canonical circadian functions. Our current hypothesis is that BcFRQ1's characteristics as an intrinsically disordered protein, drives Liquid-liquid phase separation (LLPS) modulating the activity of some of the abovementioned regulators.

Optoecology and synthetic communities (*LL*, *FC*): We are advancing the emerging field of optoecology (Rojas et al., PMID: 39912663) by integrating optogenetics into synthetic yeast communities to dissect and reprogram microbial interactions with light. Using spatially and temporally controlled illumination, we can regulate gene expression, metabolite exchange, and social behaviors such as flocculation and public goods sharing. Our current efforts involve syntrophy, engineering yeast strains that depend on each other by exchanging essential metabolites, in order to study community structure as public goods are available (light), or scarce (darkness), as we impose light regimes of different duration (**Fig. 16**). Thus, we are exploring how light-gated cooperation impacts population dynamics, allowing us also to assess Allee effects (correlation between population size

or density and the mean individual fitness of a population or species) by modulating community composition and initial cell densities, as well as spatial patterns of syntrophy. This paradigm enables us to dynamically (by imposing light regimes) shape community structure, control metabolic workflows, and assess emerging behaviors such as cheaters or cooperation.

Plant Growth-Promoting fungi (***EV***, ***LL***, ***RG***, ***PC***, ***JE***): *Penicillium yarmokense* is an endophytic fungus isolated from Solanaceae plants inhabiting the Atacama Desert. Preliminary studies in *S. lycopersicum* revealed that this strain enhances tolerance to abiotic stress. We further confirmed that *i*) it exhibits antagonistic activity against the phytopathogen *B. cinerea*, possibly via antibiosis, and *ii*) it stimulates the germination of commercial tomato seeds (cv. Roma). In addition, *P. yarmokense* significantly promotes plant growth by increasing leaf area, shoot length, and root surface area. We are currently performing functional studies to elucidate the activation of key genes under extreme conditions combined with specific abiotic cues, such as nitrogen deficiency or extended photoperiods (**Fig. 17**). In parallel, we are characterizing a second endophytic fungal isolate, initially classified as *Penicillium chrysogenum*, which also promotes plant growth. To uncover their modes of action, both fungal genomes have been sequenced and are currently undergoing functional annotation.
Similarly, we have been also analyzing the effect of a fungal isolate belonging to the *Humicolopsis* genus. The isolate, obtained from the Chilean Patagonia, promotes plant growth, including phenotypes associated with root hair length (**Fig. 18).** We have identified that the Arabidopsis master regulators RHD6 and RSL4/RSL2 are required for *Humicoplsis* induced RH growth. We are currently sequencing this fungal isolate, in order to have a reference genome and conduct dual transcriptomics, as the fungus is promoting RH growth. Importantly, our recent work highlighted RHD6 key role in the context of the early transcriptomic responses of RH cells to low temperature and nutrient availability, as inferred from GRN analyses (Urzua et al., PMID: 39891516).

(RL3) Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory

Molecular memory in response to fluctuations in nitrate availability (*RG*): We assessed whether *A. thaliana* plants have a "nutritional memory" that modulates their responses to fluctuating soil nitrate-availability. Seedlings primed with 48 hours of nitrogen (N) deficiency developed longer roots and adopted a foraging strategy upon a second N-deficiency event, unlike unprimed plants, which halted growth. This memory-like response led to increased dry weight and improved nitrogen use efficiency (NUE) in primed plants, despite no differences in total N or C content. Our findings suggest that nitrate signaling, rather than nutrient status, underlies this adaptive memory response to N fluctuations. Primed plants exhibit enhanced nitrate uptake during the recovery phase compared to unprimed plants, suggesting that priming improves nitrate absorption upon re-exposure. RNA-seq analysis revealed that primed plants upregulate key nitrate-response genes and components of the nitrate-signaling pathway during recovery, including high-affinity transporters *NRT2.4* and *NRT2.5* and their regulators. Genes involved in nitrate assimilation such as *NIA1*, *NIA2*, *GLN1.1*, *GLN1.3*, *GLN1.4*, and *GLU2*. Gene regulatory network analysis identified candidate transcription factors as potential memory regulators, and we are currently characterizing memory phenotypes in the corresponding mutants (**Fig. 19**). Overall, our results support the existence of a nutritional memory that improves plant adaptation to variable nitrate conditions.

Dynamic Metabolic Switches (*FS*, *FC*, *LL*): We engineered wine yeast strains of *S. cerevisiae* to regulate fermentation using our optogenetic switch, FUN-LOV, which enables light-controlled gene expression. We targeted the ADH1 and GPD1 genes, key regulators of ethanol and glycerol production—two metabolites that shape the sensory qualities of wine. By modulating these genes

with light, we altered metabolite balance during fermentation. Under constant illumination, GPD1 control led to increased glycerol without affecting ethanol levels. Conversely, ADH1 regulation resulted in higher glycerol production under darkness. Through growth assays and fermentation profiling, we confirmed effective optogenetic control (Ruiz et al., PMID: 39951366). In addition, also we sought to control the expression of TFs involved in fermentation, developing strains capable of switching between fermentation and respiration in response to light (**Fig. 20**). Our results highlight the feasibility of fine-tuning wine fermentation using light as a precise regulatory input.

Rewiring transcriptional programs (*LL*, *MGS*): Transcriptional rewiring enables a precise, dynamic, and programmable control of underlying gene regulatory networks (GRNs), often through the replacement of native promoters with alternative regulatory elements, proving highly effective in industrial settings, as well as for dissecting the regulatory logic of complex genetic circuits, such as the circadian clock (Goity and Larrondo PMID: 40179610). We showed that the native promoter of *frq* could be replaced by the promoter of a clock-controlled genes (*con-10*), creating a semi-synthetic or hybrid oscillator (HO-10). The latter exhibited novel properties, including a "lights-on" logic where the clock phase aligns with light onset, contrasting with the wild-type clock that resets at lights-off (Goity et al., PMID: 38627599). Building on this strategy, we also developed other semi-synthetic oscillators based on composite promoters—that is, promoters generated by combining *cis*-elements and permuting their order. The results revealed a series of unexpected findings, among which aspects related to phase determination are particularly noteworthy: we were able to observe “lights-off” and “lights-on” behaviors and, notably, intermediate types (**Fig. 21**), something not previously reported (Del Rio-Pinilla, in prep). Such SynBio strategies further confirm that transcriptional aspects of the clockwork modulate properties such as phase and amplitude, whereas period is minimally affected by this type of perturbation. Moreover, building upon previous work, we seek to delve into the selective advantages and *de novo* emergence of plasticity and oscillatory cycles in fluctuating environments., aided by mathematical modeling (Gomez-Schiavon et al., PMID: 38811585). Furthermore, we explore the interplay between these mechanisms of adaptive variation and classical genetic adaptation through mutations across various evolutionary contexts.

Reinforcing molecular cycles based on environmental fluctuations (*LL, MGS*): The heat shock response (HSR) enables cells to survive elevated temperatures by activating HSF-1, a transcription factor that induces protective heat shock proteins (HSPs). In *Neurospora crassa*, HSF-1 targets the *hsp30* promoter, which drives expression of a major HSP. Although *hsf-1* is essential, its broader roles remain understudied. Leveraging *Neurospora*’s strengths in circadian and synthetic biology, we rewired the *hsf-1* promoter to respond to light- and clock-regulation, in order to dynamically control the levels of this TF. We then compared the fitness (measured as growth rate) of a WT and rewired strain, under thermal cycles of increasing temperature, conducting an experimental evolution of multiple mitotic cycles. Importantly, the rewired strain presents an increased fitness from the first moment, whereas the WT strain “evolves” increased growth rate after ~1000 mitotic cycles, phenotype that occur in multiple pseudoreplicas (**Fig. 22)**. Once we confirmed that the phenotype was stable, we sequenced the “evolved” strain in order to find plausible causal mutations underlying the increased phenotype, analysis that is under course (Mardones et al., in prep).
We developed an electronic system based on microprocessors (see LiteCycler in RL4) that allows dynamic monitoring of the behavior of bioluminescent reporters under different light/dark cycles (alone or in conjunction with temp cycles), when using a sensitive CCD camera. This has been key to study various synthetic promoters that exhibit notable differences in their expression kinetics under environmental oscillations, with their phase of expression reveals unexpected behavior (**Fig. 23)**.

Moreover, we have observed that the behavior of these reporters (which track WCC activity), present distinct behaviors depending on how the circadian clock has been rewired.

RL4. Implementation of open source technologies and promotion of open science (*FF,PC,CR*)

***Agronomical Enabling Technologies* (*PC*):** WS-YOLO is an advanced High-Throughput Phenotyping (HTP) platform developed to detect drought-induced stress in lettuce seedlings, assuring high accuracy and speed. By integrating autonomous robotics, deep learning (YOLOv8) and infrared (IR) imaging WS-YOLO enables early stress detection (readily by day 2 of drought), allowing real-time agronomic decisions for better irrigation and water efficiency. Thus, lettuce were grown under controlled conditions, and a Raspberry Pi-based robot equipped with IR cameras collected over 2,100 images, which were categorized into four stress levels and augmented to train the WS-YOLO model using the PyTorch framework (**Fig. 24**). The model achieved strong performance, outperforming earlier models like YOLOv5 and YOLOX, particularly in early stress detection thanks to its integration with IR imaging. This technology shows strong scalability and potential for application to other crops and stress types, exemplifying how AI, robotics, and computer vision can transform precision agriculture, boosting food security and sustainability. Future work aims to extend the system to other species and integrate omics data and temporal monitoring.

Enabling bioconsumables (*FF*, *FC*, *CRS*): We have continued committed to improve access to biological reagents in LATAM, which includes the establishment of an international collaborative network of researchers who share open-access protocols, host a scientific forum, and maintain a DNA library to support local production of biologicals and freely available enzymes. This work has been recently highlighted in ***Science*** (**Fig. 25**) by an interview to FF and cofounders (https://tinyurl.com/reactivos).

This work on local biomanufacturing has also given rise to an exploration of the potential use of off-patent enzymes for commercialization. In this line, we highlight the visit of Kavi Shah (University of Cambridge, UK) to FF lab that resulted in a study of the challenges and opportunities within the biotechnology and biomanufacturing sector in South America. A white paper consolidating insights from industry stakeholders, academic experts, and research institutions across South America, has been published to serve as a valuable resource for policymakers, investors, and practitioners alike. https://zenodo.org/records/14652549.

It is also worth mentioning the work on the local production of molecular biology reagents for diagnostics (Cerda et al., PMID: 38271448) and education (Cerda et al., bioRxiv 2024.03.28.587173). This work brings costs down by one to two orders of magnitude, impacting our research and teaching considerably. Indeed, with these enabling technologies not only have we catalyzed a complete transformation of our core biochemistry courses at PUC (as a pilot initiative) but also laid the groundwork for the creation of open educational resources (OERs) targeted at high schools. This initiative aligns with PME (Proyección al Medio Externo) objectives, contributing to long-term capacity building among secondary education science teachers. Importantly, this line of work has led to the development of personalized, low-cost teaching resources**,** achieving over 95% reduction in costs for undergraduate laboratory education. In many institutions, especially in low- and middle-income countries, access to hands-on training is severely limited due to the cost of reagents and equipment.

We are making efforts to change this: for example, ***FF*** organized a workshop at the Universidad de Magallanes, training high school teachers in the use of open technologies for low-cost implementation of PCR and LAMP reactions (**Fig. 26**). Participants were introduced to cutting-edge protocols for producing enzymes at ultra-low cost using *cellular reagents*: bacterial cells expressing recombinant

proteins that can be used directly as PCR reagents, bypassing the need for expensive imported enzymes.
Thus, by combining locally produced biological reagents, open hardware, and free software, we have developed robust OERs for teaching essential biotechnological techniques, including LAMP, RT-PCR, enzyme kinetics, and fluorescence imaging. We are currently exploring partnerships with companies that support social programs, with the goal of piloting these tools in rural schools and regional universities.

Hardware (*FF*, *LL*): With respect to the goal of reducing equipment costs, we highlight the development of open source hardware dedicated to in-the-field research that has allowed the deployment of molecular diagnostic reactions in remote settings. This work involves the use of open source incubators, transilluminators, thermocyclers and colorimeters to produce enzymes from freeze-dried cell lysates transported at room temperature. This pipeline significantly reduces the logistics and costs of environmental monitoring (**Fig. 27**). While some of these open devices have been commercially acquired from open hardware companies such as IOrodeo and GaudiLabs (ie pocketPCR), the majority of devices have been developed locally in collaboration with IOWlabs (a local open hardware company) and Biofab lab UC. These tools are currently being documented to allow their reproduction and employment by other researchers. We highlight the collaboration with researchers of U. Cambridge that resulted in the development of an open source device for the incubation and real time measurement of isothermal DNA amplification reactions and cell free sensores, recently accepted in PLoS Biology (Quero et al., in press).
We also developed LightCycler (**Fig. 23**), which delivers light to Petri Plate dishes or 96-well plates. This system enables the quantification of low-intensity bioluminescence acquisition (luciferase), even under illuminated conditions, by momentarily turning off the lights while the shutter of a sensitive CCD camera is open, allowing a unique understanding of transcriptional dynamics under light-dark regimes.

iBiologist (*RG*, *EV*, *LL*): We set out to design a specialized large language model (LLM) tailored for biology, aimed at assisting users in accessing academic literature within the field. Emulating a conversational interface, the model—developed in collaboration with the National Center for Artificial Intelligence (Centro Basal CENIA)—is intended to be user-friendly, even for individuals without bioinformatics expertise. To achieve this, an MPT model with 7 billion parameters was initially trained on a diverse pretraining corpus comprising abstracts from all papers indexed in PubMed, supplemented with preprints from BioRxiv (2018–2024), content from Europe PMC, and 25% general-domain data from the CommonCrawl-based ‘The Pile’ dataset. Fine-tuning was subsequently performed using question-answer pairs derived from PubMed Q&A datasets. Despite rigorous training, the initial model exhibited suboptimal performance across multiple biological benchmarks when compared to contemporary standards. It demonstrated a general understanding of the domain but struggled with granular, domain-specific details and showed usability limitations. In response, the model was retrained adopting a more advanced architecture—GPT-2 with 1.5 billion parameters—following the BioGPT methodology pioneered by Microsoft. This iteration excluded BioRxiv and web data, focusing solely on PubMed abstracts while maintaining fine-tuning on PubMed Q&A. The refined model was rigorously evaluated on a suite of biological benchmarks, including BC5CDR (43/45), HoC (85/85), KD-DTI (35/38), and DDI (39/49), achieving performance levels consistent with state-of-the-art models. These results highlight the efficacy of the BioGPT approach and confirm the model’s capacity to deliver coherent, contextually relevant responses within the academic biological domain. Building on these advancements, the path forward involves the adoption of a 3-billion-parameter model, such as LLaMA 2, to further enhance task-specific capabilities in biology. In preparation, an alignment dataset consisting of 3,036 meticulously curated

instructions was developed in collaboration with domain experts from iBIO. This dataset was collected via a bespoke platform designed by CENIA, which facilitates unbiased model evaluation by enabling experts to interact with anonymized model outputs and select the most accurate responses based on their expertise. Additionally, ongoing efforts are focused on assembling a high-quality, well-curated corpus centered on medicine and health topics to complement existing PubMed data.

2.4 Summary table

Category of Publication [1]	**MSI Center Members**	**Number of Publications co-authored by students**	**Total Number of Publications**
WOS Publications or Similar to WOS Standard	Principal Researchers	9	17
	Other Researchers	3	24
SCOPUS Publications or Similar to SCOPUS Standard	Principal Researchers	0	0
	Other Researchers	0	0
SCIELO Publications or Similar to SCIELO Standard	Principal Researchers	0	0
	Other Researchers	0	0
Scientific Books and chapters	Principal Researchers	0	0
	Other Researchers	0	0
Other Scientific Publications	Principal Researchers	0	0
	Other Researchers	0	0
Total of Publications		**12**	**41**

3. Other relevant scientific achievements:

- *Presentación en congresos, Keynote speaker, conferencias y/o exposiciones*

PC was invited to speak at the 32nd Fungal Genetics Conference concurrent session "Connections between light, clocks, and stress" (California, USA).
FC presented at the 16th International Congress on Yeasts in South Africa and the EMBO Workshop: Molecular Mechanisms in Evolution and Ecology in Germany.
JE presented at *Plant Biology 2024 Virtual Workshops & Extended Sessions*, an extension of the prestigious Plant Biology 2024, organized by the American Society of Plant Biologists (ASPB).
FS was invited to speak at the VII Reunión del Grupo Argentino de Fotobiología (GRAFOB) (San Luis, Argentina)
RG was invited to give a plenary talk at a Salt and Water Stress Gordon Research Conference (USA), at the first Plant Resilience Summit (USA), and the Stress UNIA International Workshop in Baeza (Spain) among other scientific talks.
LL was invited to give plenary talks at the Argentinean Society for General Microbiology (SAMIGE), Brazilian Society of Genetics (SBG), and the EMBL Symposium- Biological oscillators- rhythms and synchronization across scales (Germany).
CRS presented at the EMBO 60th Anniversary Meeting, Heidelberg, Germany (2024) "Discovery and engineering of PET hydrolases: enzymatic marvels unleashed"
EV was an invited speaker in the "Plant Systems Biology: from molecules to the ecosystem" symposium during the XLVII Reunión Anual 2024 de la Sociedad de Bioquímica y Biología Molecular de Chile

- *Organización de eventos científicos*

MGS co-organized the Biomathematics Workshop as part of the *Escuela Queretana de Matemáticas*, held at the Instituto de Matemáticas, Unidad Juriquilla, UNAM.
JE organized an advanced course in molecular biology and plant biotechnology from May 8 to 12. The course was initially aimed at PhD students, but due to high demand, it was opened to research assistants, undergraduate students, and laboratory technicians, with over 70 participants.
EV and ***JMA*** organized a Single Cell Genomics Seminar/Workshop "Claves de la vida a nivel celular" (Santiago, Chile).
JE was also part of the organizing committee of the 8th International Conference on Plant Cell Wall Biology (Talca-Chile), and *"Plants under Environmental Stress: Overcoming Current Climate Challenges,"* in Baeza, Spain.
JC hosted a seminars series on "Plant Stress Responses and Molecular Mechanisms" at U. Austral (Valdivia)
CRS organized the "1st Symposium on Green Chemistry and Sustainability (Santiago, Chile)
Also, graduate students Cyndi Tabilo and Valeria Eltit (CRS, FF labs) organized the Workshop Predicting and designing proteins of the future using Rosetta and Artificial Intelligence", between August 5-7, 2025, in the Department of Computer Science at UC, Santiago, Chile).
LL is part of the Fungal Genetics Policy Committee, which oversaw the organization of 32nd Fungal Genetics Conference (California, USA).

During 2024 ***LL*** and ***PC***, obtained grants from UNU-BIOLAC, the Company of Biologists, and IUBMB, to fund part of the scientific program of the upcoming Molecular Biosystems Conference 2025 (https://www.molbiosystems.com/)
The work on open science policies has also led to an invitation-only participation of ***FF*** in the landmark conference Spirit of Asilomar, a conference celebrated on the 50th anniversary of the 1975 international meeting on recombinant DNA molecules at Asilomar (https://www.spiritofasilomar.org/). ***FF*** provided reflections on the relevance of open science policies and education on open source technology. Along those lines ***LL*** participated in the Focus Group Expert: OECD Global Forum on Technology (GFTech- SynBio).

- *Patentes*

Nothing to report

- *Propiedad Intelectual*

Nothing to report

- *Comités editoriales*

PC participates as an editor of the Research Topic in Frontiers in Plant Science
FC is an Associated Editor of Yeast and FEMS Yeast Research journals
JE is Associate Editor in Frontiers in Plant Sciences.***,*** New Phytologist, BMC Plant Biology, and Communications Biology
CR is an Editorial Board Member of Microbial Biotechnologies, and executive Councilor of The Protein Society, a Chilean Member of the Latin American Federation of Biophysical Societies (LaFEBS) and a Chilean Node Member of the Center for Structural Biology of the Mercosur (CEBEM).
LL serves on the editorial boards of *Scientific Reports*, *Fungal Biology and Biotechnology*, *Biological Research*, *Frontiers in Microbiology*, *Infection & Immunity*, and *npj Biological Timing and Sleep*. Additionally, he is a member of the advisory editorial board of *Genetics & Genomics Next* and part of the board of reviewing editors for *eLife*.
RG is associate editor of Molecular Plant.
FF Is part of the PLOS Board of Directors, and a member of the IBSP UNESCO, providing advice to the general director. In this context, FF was also invited, along with 19 other international scientists, to the International Scientific Board meeting of UNESCO, convened at the International Center for Theoretical Physics in Trieste (Italy) to discuss the role of basic science in the development of member states.
RN is an Associate editor in Biology Letters (Royal Society Journals) and Ecology & Evolution

- *Premios al centro y/o a sus Investigadores(as)*

Dr. **Alejandra Goity** (postdoc) received a Gold ASM award for her presentation (summarizing her collaborative work of the ***RG*** and ***LL*** labs), at the V International Symposium on Fungal Stress conference (Brasil). At the same meeting the undergraduate student Isabella Bresciani (**LL** lab) received a “Best Poster award”
At the annual meeting of the Society for Biochemistry and Molecular Biology Liliana Lamig (premio and Valentina Núñez (both grad students at the ***RG*** lab) received award for best oral presentations.

Catalina Muñoz, undergraduate (***FC*** and ***LL*** labs), received an Honorable Mention for her Oral Communication at the XLVI Chilean Congress of Microbiology..
MGS was awarded the College for Life Sciences Fellowship at the Wissenschaftskolleg zu Berlin, Institute for Advanced Study, in Berlin, Germany (August-December). This prestigious fellowship supported outstanding early-career researchers in the life sciences and medicine, providing a unique opportunity to step back from daily lab routines and engage in interdisciplinary dialogue.
JMA was honored as 2024 Researcher by the Faculty of Life Sciences of Universidad Nacional Andrés Bello (Award granted on January 22, 2025), recognizing him as an Outstanding Researcher.
CR**,** was awarded as a TED Fellow 2025 in December 2024, with a news release coming up in March, 2025.
RG was appointed Associate Member of EMBO (the third Chilean to receive this distinction)
LL was appointed Corresponding Member of the Chilean Academy of Sciences.
LL was appointed Distinguished Visiting Professor at the University of Macau, where he carried out a one-month academic visit.

4. Education and Capacity Building

Education, Training and Capacity Building:

MSI RESEARCHER	NUMBER									TOTAL NUMBER PER MSI RESEARCHER
	Undergraduate students			Graduate students						
				Masters			Doctoral			
	F	M	ND	F	M	ND	F	M	ND	TOTAL
Rodrigo Gutiérrez Ilabaca	**0**	**3**		**0**	**0**		**4**	**3**		**10**
Francisco Cubillos Riffo Roberto Nespolo Rossi	**0**	**0**		**0**	**0**		**0**	**1**		**1**
Francisco Cubillos Riffo	**4**	**6**		**1**	**1**		**2**	**3**		**17**
Francisco Salinas Sanhueza	**2**	**0**		**0**	**0**		**1**	**3**		**6**
María Francisca Blanco Herrera	**2**	**0**		**0**	**0**		**0**	**1**		**3**
Paulo Canessa Aguila	**3**	**6**		**0**	**0**		**2**	**2**		**13**
Jose Manuel Estevez Lopez	**0**	**0**		**0**	**0**		**4**	**2**		**6**
Fernan Federici Noe	**1**	**1**		**0**	**0**		**1**	**1**		**4**
Luis Larrondo Castro	**5**	**4**		**0**	**0**		**1**	**3**		**13**
Elena Vidal Olate Jose Miguel Alvarez Herrera	**0**	**0**		**0**	**0**		**0**	**1**		**1**
Cesar Antonio Ramírez Sarmiento	**3**	**0**		**0**	**0**		**2**	**1**		**6**
Javier Canales Carrasco	**0**	**1**		**0**	**0**		**0**	**0**		**1**
Elena Vidal Olate	**1**	**1**		**0**	**0**		**1**	**1**		**4**
Jose Miguel Alvarez Herrera	**1**	**1**		**0**	**2**		**0**	**2**		**6**
Eleodoro Riveras Hernandez Rodrigo Gutiérrez Ilabaca	**1**	**0**		**0**	**0**		**0**	**0**		**1**
TOTAL	**23**	**23**		**1**	**3**		**18**	**24**		**92**

iBio researchers continue to be actively involved in undergraduate and graduate programs at their host institutions, teaching a broad range of topics including biology, biochemistry, genomics,

bioinformatics, synthetic biology, and biotechnology. Beyond formal teaching, we continue to implement specialized workshops aimed at training both iBio members and external researchers in areas such as open technologies, bioinformatics, mathematical modeling, science communication, and research management. In addition, we have organized national and international workshops, with a particular focus on engaging both undergraduate and graduate students.
iBio PIs also play key roles in shaping academic curricula at their institutions. For example, ***RG*** is leading the strategic development plan for the School of Biological Sciences at PUC, while ***LL*** at PUC, ***JC*** at Universidad Austral, and ***EV*** at Universidad Mayor, are also actively involved in defining the new structures of the corresponding Ph.D. programs. We have also contributed to academic innovation by proposing team-taught courses in emerging areas such as biofabrication (***LL*** and ***FF***), in collaboration with the Schools of Design and Engineering at PUC.
In addition, areas such as mathematical modeling promote a multidisciplinary training environment that integrates theoretical, computational, and quantitative approaches. ***MGS*** teaches Systems Biology at the undergraduate level at UNAM and also serves as an instructor in UNAM's Summer School of Mathematics for undergraduate and graduate students.
At PUC, where ***RG***, ***LL***, and ***FF*** are based, the former departmental structure was recently replaced by broader thematic areas. As a result, two new academic areas were established: Biomedicine and Integrative Biology. Within these, new research programs have emerged, and we are actively involved in three of them: *i*) Plant Systems—Evolution and Resilience, *ii*) Microbial Ecology of the Biosphere, and *iii*) Theoretical Biology and Complex Systems. We anticipate that these initiatives will foster the development of new team-taught courses, with a integrative-centered perspective, as well as co-advised student projects.
Finally, iBio PIs participate in diverse Ph.D. selection committees and remain vigilant in promoting gender equity throughout academic processes. Notably, gender representation is generally balanced at the undergraduate, graduate, and postdoctoral levels across our institutions. However, disparities tend to emerge at the assistant professor level—an issue that has been acknowledged and raised with university leadership.

The work on cell-free systems and open-source enzymes has continued to attract early-career scientists from abroad to train in the FF lab. For instance, with support from a United Nations University BIOLAC grant, **Matías Villarroel** (Instituto Leloir, Argentina) joined the lab for three months to work on DNA assembly methods for biosensor engineering. Similarly, **María Fernanda Mendoza** (Universidad de Guanajuato, Mexico) trained at iBio in cell-free reactions as part of an exchange coordinated with the Eleazar Barboza lab.
A key aspect of all these initiatives is that **students have been the driving force**, dedicating their time to workshops, trainings, and collaborative activities, advancing this technological-educational agenda.
We have a vibrant scientific environment with international students visiting iBio, as well as our students going abroad to conduct internships (see Annex 4.1 and 4.2 for full details). Thus, for example, among the **incoming Students** we have**:**
Adrián González, a PhD student at CBGP (Spain), worked for 3 months at the interface of the ***RG*** and ***LL*** labs on plant-growth-promoting traits of a *Penicillium* isolate.
Gabriel Couillaud, a Master's student from Wageningen University (Netherlands), conducted a 7-month internship under the supervision of Consuelo Olivares (Young Investigator, iBio), bridging work between the ***PC*** and ***LL*** labs and establishing a TurboID protocol in *B. cinerea*.
Gustavo Husein, a PhD student from Brazil, spent 6 months in the ***JC*** lab at Universidad Austral (August 2024), focusing on transcriptomic and metabolomic analyses of the soybean–rust pathosystem under water deficit.

Sergio Izquierdo, a PhD student from Universidad Complutense de Madrid (Spain), worked for 3 months in the ***FF*** lab, conducting genomic analyses of *Yarrowia lipolytica*.
Nina Vittorelli, a PhD student from Université Sorbonne (France), spent 6 weeks in the ***FC*** lab, analyzing alpha-factor responses in native yeast and their mating behavior.
Macarena Mellado, a PhD student from CSIC-UPV (Spain), developed *Marchantia polymorpha* experiments during her 2-month stay in the ***RG*** lab.
María Fernanda Mendoza, a PhD student from Universidad de Guanajuato (Mexico), worked on cell-free reactions at the ***FF*** lab for 7 weeks.
Matías Villarroel, from Instituto Leloir (Argentina), worked for 3 months in the ***FF*** lab on DNA assembly methods.
Kavi Shah, a PhD student from the University of Cambridge (UK), conducted a 6-week qualitative study on biotech in Latin America while based at the ***FF*** Lab.
Marc Dusseiller, a postdoctoral researcher with Hackteria (Sweden), engaged in art-science projects over a 3-month stay in the ***FF*** lab.
Amélie Malleville, a Master's student from Université de Montpellier (France), conducted a 3-week project on *Hoffmanseggia doelli* in the ***RG*** lab.

In 2024, a total of 46 undergraduate students, 46 graduate students, 21 postdoctoral researchers, and 12 Young Investigators carried out research in iBio-affiliated laboratories. Among them, 10 undergraduate and 6 graduate students (2 Ph.D. and 4 Master's students) successfully completed their academic programs. The accompanying table in this section summarizes the current academic and professional status of students across the different labs.

We are pleased to note that, as in previous years, many of our graduates have chosen to continue on academic paths, often staying in iBio labs. We see this as a strong indicator that our training environment fosters a passion for science and is valued by students as a stimulating -and safe- space for continued learning and research. At the same time, we are equally proud to see some students pursuing careers in the private and productive sectors, as well as gaining acceptance into Ph.D. programs abroad—such as the University of Edinburgh—and at institutions within Chile.
A notable highlight this year was the graduation of the first two Ph.D. students from the ***PC*** Lab, marking a major milestone in the PC lab's history and its contribution to advanced scientific training.

Throughout the year, our young researchers engaged actively with Associate Investigators and collaborators across disciplines and institutions. They played central roles in experimental design, data analysis, and manuscript preparation. To further support their development, we organized a variety of workshops, courses, and seminars on topics such as molecular biology, bioinformatics, and plant physiology. These efforts reflect our ongoing commitment to providing a comprehensive and high-quality training environment that equips the next generation of scientists with the skills and experience needed for successful research careers

In 2024, iBio signed a Memorandum of Understanding (MoU) with the Fundación Ciencia y Vida (FCV), which is associated with Universidad San Sebastián, to strengthen ongoing academic and technological initiatives in biotechnology. A key objective of this agreement is to provide iBio's young researchers and postdoctoral fellows with opportunities to gain teaching experience, while allowing FCV to explore new areas of research expansion in collaboration with iBio younger and future investigators.
Our alumni have continued to secure impactful and fulfilling career opportunities, both in academia and industry. For example, former PhD student Camila Baeza (***FS*** Lab) is now a postdoctoral

researcher at CNRS in Marseille, France, where she works alongside former iBio postdoc Vicente Rojas (***LL*** Lab), who also started a postdoc at CNRS. Vasni Zabaleta, who was completing his PhD in the ***FC*** Lab, has now joined the ***LL*** Lab to start as a postdoctoral fellow. Kamila Fernández (***PC*** Lab) transitioned into the biotechnology sector, joining a biotech company shortly after obtaining her Ph.D. At the undergraduate and technical levels, several individuals have chosen to pursue Ph.D. programs, often remaining affiliated with iBio labs, while others have moved abroad to continue their academic training (i.e. Isabella Bresciani (***LL*** Lab) was accepted for her PhD studies at the University of Edinburgh).

The iBio institutional budget and infrastructure have also played a critical role in supporting early-career researchers—especially those not yet formally affiliated with a university. For instance, **Consuelo Olivares-Yáñez**, a Young Investigator in the ***PC*** Lab, was appointed Assistant Professor (tenure-track) at Universidad Mayor in 2024, where she is now establishing her independent research group.

In the same year, **Wladimir Mardones** (***LL*** Lab) and **Pablo Villarreal** (***FC*** Lab) were both awarded FONDECYT-Inicio grants, enabling them to fund their own independent research projects. These achievements have significantly enhanced their academic profiles, positioning them competitively in the Chilean academic job market. Both have been invited for interviews at leading universities across the country. We are currently supporting additional Young Investigators on the iBio payroll who are in the process of applying for independent funding and academic positions.

5. Collaborative networks

a) *Formal Collaboration Network Plan*:

The iBio has rarely, as a center, signed formal collaboration agreements. Instead, our collaborative framework is built on a robust network of partnerships established through the PIs affiliated with the Institute. These collaborations range from long-standing, deeply integrated relationships to emerging partnerships, many of which are a result of the growing international recognition of our members.
Importantly, to achieve and sustain our scientific goals, we emphasize both international and national collaboration. As noted previously, each PI maintains active networks of internal and external collaborators, which in turn enrich the iBio ecosystem through a wide range of activities—such as workshops, seminars, seminar series, and research internships. These collaborative efforts contribute to a dynamic and intellectually stimulating environment, significantly broadening the academic and professional horizons of our students.
That said, in 2024 iBio has formally established only three collaboration agreements as an entity, as detailed below:
CENIA: Led by the ***RG*** Lab, this partnership with the National Center for Artificial Intelligence (CENIA, Chile) was launched at the end of 2023. The collaboration aims to co-develop a specialized large language model (LLM) for biology, designed to enhance access to academic literature, generate hypothesis-driven insights, and optimize experimental design. Throughout 2024, several follow-up meetings were held to assess project progress and address key challenges.
Fundación Ciencia y Vida (FCV): As mentioned earlier, a Memorandum of Understanding was signed between iBio and FCV with the goal of expanding FCV's capabilities in biotechnology. This agreement creates opportunities for our young investigators to gain teaching and mentoring experience, while potentially contributing to new research areas within FCV. Additionally, FCV offers valuable expertise and legal infrastructure in technology transfer, which has the potential to support and scale emerging spin-offs from within the iBio community.
RIKEN: Led by the ***RG*** Lab, this partnership with the RIKEN Center for Sustainable Resource Science in Japan, was launched at the end of 2024. The collaboration aims to apply existing technology for drought and heat stress to improve perennial crop yield.

b) *State of progress and readjustment*:

As commented above, collaborations with CENIA and FCV are ongoing experiences and we foresee no immediate readjustment.
We would also like to mention another collaboration with **FADEU–PUC**, which has gained additional momentum during 2024. Over the years, we have established a vibrant collaboration with colleagues from the Art and Design Schools at PUC in the development of fungal-based biomaterials (led by ***FF*** and ***LL***). In 2024, this interdisciplinary collaboration expanded to include the School of Engineering, as ***LL*** is co-supervising a graduate student, Hugo Muñoz (from the Civil Engineering Ph.D. program), alongside Professor Magdalena Wolzack. Hugo is based at FADEU, working in the BioFab Lab, an initiative championed by our collaborator, Professor Francisco Chateau.Notably, his efforts led to, in 2024, to the inauguration of a new and upgraded BioFab Lab, providing enhanced facilities for interdisciplinary work. In parallel, we have been developing a new course on biomaterials to be offered at PUC, and we are actively preparing grant applications to support further research and innovation in this area.

c) ***Main achievements***:

FF and ***CRS*** secured international funds from CYTED for the employment of open source technologies in R&D in Latin America. The collaborative network, called Red Latinoamericana de Reactivos Abiertos para Una Salud (RELARUS), is led by ***FF*** and will work with researchers from Cuba, Brazil, Argentina, Colombia, Peru and Uruguay for 4 years.

FF, founder of Reclone, the open reagents collaboration network, teamed up with ***CRS*** and researchers from UNCuyo (Argentina) & UPCH (Peru) to secure funding from the Chan Zuckerberg Initiative to foster the access to public domain biological reagents in Peru, Chile and Argentina.

During 2024 ***EV*** participated as Director of "ResilomicsNet: Establishment of an international alliance to potentiate climate resilience in species of productive interest- FOVI230159", funded by "Concurso de Fomento a la Vinculación Internacional para Instituciones de Investigación-ANID" (2023-2024).. The network includes researchers in U. de Santiago de Chile (Dr. Felipe Reyes, Dr. Mónica Imarai), U. Andrés Bello (Dr. José Álvarez-iBio), U. Austral (Dr. Javier Canales-iBio), U. de O' Higgins (Dr. Carol Moraga-iBio) and U. Mayor (Dr. Sebastián Reyes, Dr. Andrea Miyasaka, Dr. Carlos Maldonado) and international collaborators in the I2SysBio, U. de Valencia, Spain (Dr. Tomás Matus), Center for Plant Biotechnology and Genomics, Madrid, Spain (Dr. Joaquín Medina) and U. Autónoma de Barcelona (Dr. Lluis Tort). In 2024 the network organized different seminars and workshops focused on plant genomics and biotechnology, with the participation of the international researchers, who travelled to Chile during October.

At the national level ***EV*** acts as Deputy Director of the Millennium Nucleus in Data Science for Plant Resilience Phytolearning, a project focused in understanding how plants balance N and water signals for optimizing growth and expands her collaborative network to the other PIs of the nucleus Dr. José Álvarez (UNAB), Dr. Gonzalo Ruz (UAI), Dr. Romina Sepúlveda (UNAB), Dr. Juan Pablo Vasconez (UNAB), Dr. Claudio Inostroza, (UCM-Temuco), Dr. Carol Moraga, (UOH-Rancagua) and Dr. Mabel Vidal (U. Concepción).

JMA has participated as a Co-Principal Investigator in a National Science Foundation grant (NSF 1840761); led by Dr. Coruzzi, (New York University, USA) that focuses on elucidating how environmental signals shape plant physiology and agricultural outcomes.

RG also has strong collaborations with the Max Planck Institute of Molecular Plant Physiology in Golm, Germany. In addition, we continue collaborating with Dr. Coruzzi and colleagues from the New York Botanical Garden and the American Museum of Natural History, thereby reinforcing a robust network of interdisciplinary and inter-institutional scientific exchange.

FC maintains active collaborative networks with researchers both in Chile and abroad. Among its international collaborators are: **Diego Libkind** (IPATEC, Argentina), **Chris Hittinger** (University of Wisconsin–Madison, USA), **Gianni Liti** (Université Côte d'Azur, Nice, France), **Gilles Fischer** (Sorbonne Université, France), **Rike Stelkens** (Stockholm University, Sweden), **Ignacio Belda** (Complutense University of Madrid, Spain), and **Amparo Querol** (Institute of Agrochemistry and Food Technology, Valencia, Spain).

JC has multiple international collaborations, such as **Dr. Joaquín Medina** (CBGP, Spain), focused in uncovering plant responses to sulfate starvation, with **Dr. Daniel Calderini** (Universidad Austral de Chile, Valdivia), focusing on improving wheat yield and adaptability, and **Dr. Cristóbal Uauy** (John Innes Centre, UK), related to wheat genomics.

LL also has involved multiple international collaborations., such as **Dr. Reinhard Fischer** (Karlsruhe Institute of Technology, Germany), studying the circadian clock of *A. alternata*., **Dr. Carrie Partch** (UC Santa Cruz, USA) on the structural and functional analysis of Casein Kinase I (CK1), **Dr. Jay C. Dunlap**, Dartmouth College, on clock molecular mechanisms), and **Chris Hong**, on mathematical modeling.

Among other new collaborations established during 2024 are:
RG began a new project with his long-standing collaborator **Dr. Laurence Lejay** (INRAE, France) and **Interstellar Lab**, a French startup focused on space-agriculture technologies. The collaboration explores how plants sense and respond to nutrient availability in controlled environments, including extraterrestrial settings, aiming to uncover the mechanisms of plant adaptation and resilience under extreme conditions, both on Earth and in space.
CRS signed a **Non-Disclosure Agreement (NDA)** with **AI Proteins Inc.**, a Boston-based biotech company specializing in the design of miniprotein binders with pharmaceutical relevance. This agreement enabled the launch of internship opportunities between students in the CRS lab and AI Proteins, fostering industry-academic exchange in protein engineering.
LL initiated a collaboration with **Dr. Vayu Hill-Maini at Stanford Bio-X (USA)** to investigate ways of enhancing *Neurospora*'s capacity to grow on agro-industrial plant waste. The aim is to upcycle biomass into novel fermented products, contributing to circular bioeconomy strategies. ***LL*** also established a new partnership with **Dr. Chris Wong** (University of Macau), which included a one-month research visit. This collaboration focuses on transcriptional regulation in fungi, particularly the role of liquid-liquid phase separation (LLPS) dynamics. Additionally, ***LL*** started a research collaboration with **Dr. Ilkka Kronholm** (University of Jyväskylä, Finland), centered on thermotolerance and experimental evolution in fungi, expanding the lab's focus on adaptive mechanisms under stress conditions.

d) ***Leverage of resources*****:**.

Through various funding mechanisms, we have been able to allocate resources to strengthen these collaborations. In particular, student internships can often be supported through *fondos complementarios* linked to their graduate ANID fellowships. However, sustaining this model is becoming increasingly challenging due to two main factors: i) a decline in the availability of external funding, and, ii) rising financial requirements imposed by host institutions. For example, the University of California system now mandates that visiting graduate students receive a minimum monthly stipend of USD 3000, while ANID fellowships typically provide only ~USD 1500/month, creating a significant funding gap that limits access to such opportunities.

6. Outreach and connections with other sectors

a) Outreach and Projection to the External Environment:

During 2024, the main outreach project was aligned with one of the core objectives of our center: to promote the use of Open Science tools in education and research. Specifically, we focused on the development and implementation of open-source scientific instruments, free software, and locally produced biotechnological reagents. The project's main goal is to train high school teachers in the use of Open Educational Resources (OER) that integrate open hardware, free software, and freely accessible biotechnological reagents for the hands-on teaching of molecular biology.

It is important to highlight that, due to ANID restrictions, it was not possible for existing Institutes to apply for PME (Proyeccion al Medio Externo) funds to be executed in 2024. Still, we managed to devote resources from our central funds to execute activities we deem necessary.

Through our Millennium initiative, over the years we have developed:
- Low-cost, open-access scientific instruments for molecular biology experiments, such as DNA/RNA amplification and fluorescence detection.

- Open-source digital notebooks (Jupyter Notebooks/Google Colabs) that require no installation or dependencies, enabling immediate online use and making them accessible to beginners.

- Locally produced enzymes, buffers, and public-domain reagents, suitable for conducting molecular reactions such as LAMP and PCR for both educational and research purposes.

In collaboration with several partners, we have created a “portable lab kit” that integrates open-source instruments and molecular biology techniques, allowing the performance of PCR and the identification of transgenic genes via LAMP, all at very low cost.

Although these techniques have great potential in molecular biology and the detection of organisms of interest (e.g., pathogens or GMOs), practical instruction is often limited by the high cost of equipment and reagents. Our center is actively working to reduce the complexity, dependencies, and costs associated with these methods, in order to expand access to schools and universities.

These resources enable interdisciplinary learning, combining basic electronics, 3D design and printing, optics, molecular biology, and programming. Moreover, using open and well-documented devices helps avoid the “black box” effect imposed by proprietary and closed equipment, which can hinder education by obscuring the fundamental principles behind the operation of scientific tools.

We trained teachers in PCR and LAMP, combining the use of very low-cost equipment (USD 100 for a PCR thermocycler or USD 250 for a LAMP incubator) with the local production of necessary reagents. In this way, we focus on two main limitations: First, we address the limited access to equipment by enabling the fabrication or commercial acquisition of low-cost, open-source devices, which are currently produced and sold in Chile by the company IOWlabs (e.g. low cost transilluminator). This company has collaborated with our center for four years in the development of scientific instruments that can be commercialized or reproduced by third parties. This collaboration represents a new model of technology transfer, promoting public-private cooperation and open innovation. This partnership allows us to offer affordable solutions to teachers with limited resources,

thereby avoiding the need to purchase closed and expensive instruments (i.e. ~USD 4000 for a PCR machine, or USD 1500 for a commercial transilluminator), achieving at least an order-of-magnitude reduction in equipment costs.

Second, we offer a potential solution to the issue of reagent access. Access to enzymes and buffers remains a significant barrier, as these reagents are expensive and must be imported under cold chain conditions from the United States, which greatly limits the teaching of these techniques in high school classrooms.

We have addressed this issue by establishing local enzyme production protocols, optimized to reduce costs and infrastructure dependencies. For example, local production of enzymes and extracts has decreased the cost of biological reagents by one to two orders of magnitude. However, these techniques typically require a well-equipped laboratory (shakers, incubators, centrifuges, etc.).
Recently, in collaboration with the Open Bioeconomy Lab (University of Cambridge), we have begun exploring low-cost methods for producing these reagents without relying on such infrastructure. For instance, we are producing enzymes in small plates or tubes incubated on a heated bed, instead of in liquid cultures using shakers and incubators.
Although these reagents are <u>not</u> (yet) suitable for research purposes due to their variability, they do offer a viable solution for accessing reagents in educational settings. Therefore, we plan to train teachers in various low-cost enzyme production techniques, with the goal of exploring their implementation in schools during a second phase (Outreach application, 2026). In addition (see coming section), one of the iBio PIs (***CRS***), has funded a startup that will help bridging this gap.

b) **Connections with other sectors:**

<u>Companies and Private Sector</u>

We continued our work with *Cultiva*, a Nevada-based (USA) biotechnology company, which is providing funds to study an agrochemical product (commercialized in Chile), analyzing its potential to control *B. cinerea*. The **PC** lab has confirmed that the product affects the ability of the fungus to colonize the plant, yet it does not block spore germination.

During 2024, we were approached by Bioforest, a local Chilean owned biotechnology company that is interested in using different fungal species in the pulp and paper industry. We planned different experiments but no formal contract or monetary contributions have been received. Likewise, during the past year we were approached by Tattersall (Agro-division), so we could provide expert support in the evaluation of different agronomic products. We expect this agreement to be formalized during 2025.

CRS co-founded a startup company in October 2024, NexEnzymes for the design of molecular kits for PCR, qRT-PCR, alongside Bernardo Collao (CEO) and Daniel Almonacid (CFO). This new venture also includes consultancy services from our research group at UC to NexEnzymes, hiring Ph.D. students in part-time allocations for the development of these kits.

RG and ***FC*** made significant progress on the FONDEF IdeA project “Endemic Whisky,” which is produced exclusively using national raw materials. This experimental product was presented at international conferences in 2024 and received highly positive feedback from attendees. We also

performed two tasting events with professional tasters as well as whisky enthusiasts receiving top marks compared to high end commercial whisky. This project is possible by the combined expertise in fungal biology (***FC***) and plant biology (***RG***) within iBio and the collaboration with the distillery Esenlid led by Master Distiller Tomas Schaerer. During 2024, we secured funding from two additional government agencies to create a spinoff company and to scale up production of consumables used in our whisky making. Moreover, we applied for additional funding programs to address and optimize specific problems of the whisky production process.

ONGs and Social Organizations

EV and her group continues to collaborate with ONG Susténtate, helping in the organization of the Academia de Biotecnología Agrícola (ABA), a workshop that trains women school students from rural sectors of Chile in microbiology and molecular biology laboratory techniques for plant biology, project writing, leadership and entrepreneurship. ***EV***'s lab helps in the preparation of Laboratory kits for distribution in different localities in Chile, and with inspirational talks from women in science.

EV has started a collaboration with small farmers from Calle Larga, Valparaíso, in order to determine the microbial components of biofertilizers produced by the community. We visited farmers during December and obtained samples of biofertilizers of different compositions. We are currently isolating bacterial and fungal growth in these biofertilizers to characterize them molecularly, and gain insights into their plant growth promoting properties. We are also investigating the effect at the molecular level of these biofertilizers on tomato plants. We hope this knowledge will help farmers to promote their products and also to define specific preparations that work better to boost plant production. We worked on this project with Maite Salazar, who is in charge of outreach projects in iBio, and has worked with these communities for several years.

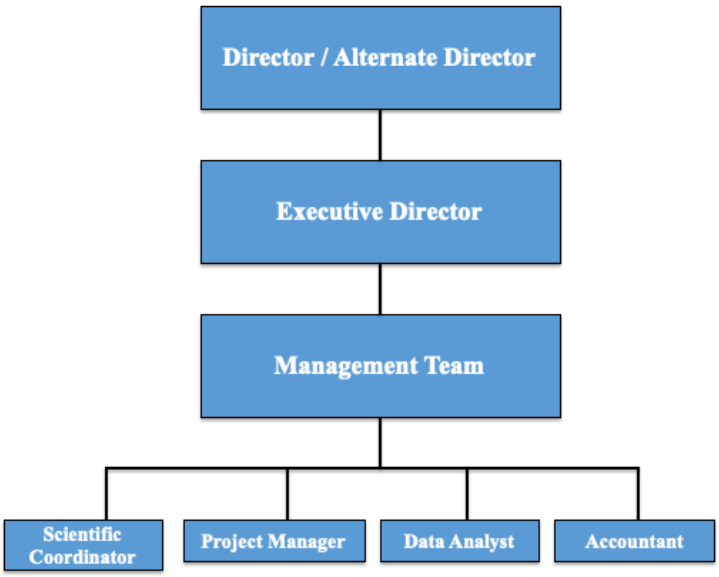
We were also involved in promoting interactive science activities and talks for high school students, such as theLiceo Bicentenario Hualañé in the Maule Region, exposing them to the bases of molecular biology and biotechnology.

Artistic Interventions

Work by the ***FF*** lab was part of the Impronte exhibition, organized by the University of Parma, and Municipality of Parma and the Botanical Gardens of Padua from January to April 2024. reaching over 21000 people in Parma, 257 publications in newspapers, magazines and websites, and seven separate television and radio reports. Given the success of the exhibition, it was later hosted in the Botanical Gardens, an Unesco World Heritage Site, as well as at the Palazzo Eccheli-Baisi in Brentonico, in collaboration with the Fondazione Museo storico del Trentino, https://museostorico.it/exhibition/impronte-noi-e-le-piante/
A book was also released: https://www.edizioniets.com/scheda.asp?n=9788846768506#tab2
As part of a ProHelvetia grant, Marc Dusseiller, from the citizen lab Hackteria (Switzerland) visited **FF** to do artistic workshops (e.g. Biofab UC & UNCuyo Mendoza) and visits to interact with local artistes (e.g. Balmaceda arte joven, Santiago & LabVa citizen lab, Valdivia)).
https://federicilab.org/2024/10/29/taller-de-tecnologias-abiertas-open-hardware-cell-free-open-enzymes-en-uncuyo-mendoza-junto-a-hackteria/
FB led the large-scale interactive exhibition *"Rizosfera Viviente"*, engaging over 500 members of the general public on the importance of plant-microbe interactions in the context of climate change and sustainable agriculture.

7. Administration, Governance and Financial Status

Organization and administration:



The “Fundación Instituto de Biología Integrativa” is the host institution of the Millennium Institute iBio. Over the past year, the Foundation's Board met three times to review the institute’s general progress and discuss its strategic direction. Weekly meetings among the Associated Investigators remain a core practice, serving to coordinate and guide initiatives in science, outreach, administration, recruitment, and other key areas of iBio's operations.

Our decision-making is grounded in a horizontal, collaborative ethos that reflects our commitment to a participatory and inclusive institutional culture.

In 2024, our executive team was led by Executive Director Carolina Carriel, a biotechnologist with a MSc and extensive experience in science and technology transfer. The team also includes Scientific Coordinator Karem Tamayo, a biologist responsible for organizing scientific activities, gathering institutional data, and supporting reporting processes; and Administrative Coordinator Carolina Ramos, a business administrator who manages payments and prepares monthly financial reports.

This core team is supported by a new communications agency, whose excellent work has significantly increased our media presence and visibility in the national press. We also benefit from the legal counsel of Guido Aguirre, a lawyer with vast experience in non-profit organizations and research institutions, and from the financial expertise of accountant Pedro Bahamondes.

We remain firmly committed to combating the precarious working conditions often faced by young scientists, and we continue to take every possible measure to ensure fair and dignified employment within our institution.

La información cuantitativa de los ayudantes y técnicos, y personal administrativo que han trabajado durante el período deberá ser presentada en la siguiente tabla:

Category	Female	Male	TOTAL
Assistant & Technicians	9	7	16
Administrative Staff	5	0	5
TOTAL	**14**	**7**	**21**

b) Financial Status:

Expenses	Amount (USD)
Salaries	196.072
Fees, Wages, Scholarships, and Others	327.527
Travel, Per Diems, Conferences, Seminars	58.162
Consumable Materials	140.747
Administrative Expenses	28.689
Policy or Other Instrument Expenses	584
Visiting Professors and Consulting Services	65.358
Books, Publications and Subscriptions	14.864
Investment Expenses	33.629
Infrastructure	3.272
General Expenses of Host Institutions	26.866
Relocation Expenses	8.274
Food Expenses	5.422
Equipment Maintenance	728
Total	910.214

Total Funding:[1]

Funds	2024 Sources of Funding	
	Amount [$]	Percentage of resources used by the Center [%]
ANID - Milenio	$957.000.000	55,24%
FONDECYT	$537.550.356	31,03%
Otros Fondos Internacionales	$99.775.760	5,76%
Otros Fondos Sector Privado	$10.100.000	0,58%
Beca Otros	$28.000.000	1,62%
ANID - Otro	$100.000.000	5,77%
TOTAL	$1.732.426.116	

2024 PAUR distribution:

Type of Expense	Total Amount [$]
Scientific Staff	$ 281.828.229
Technical Staff	$ 148.385.097
Administrative Staff	$ 58.675.892
Communications Staff	$ 2.150.000
Investments	$ 33.009.518
Infrastructure	$ 787.970
Consulting	$ 64.287.094

[1] Para aquellas fuentes de financiamiento con el mismo origen, se debe realizar sólo un ingreso con el valor total de la fuente de financiamiento y no ingresar uno a uno estos montos.

8. **Other achievements [OPTIONAL]:**

9. Negative or positive aspects that you would like to address in order to understand the context in which the center developed its work during the reported period

a. Investigación científica
b. Formación de jóvenes
c. Actividades de extensión
d. Redes de colaboración

Relevant Positive and Negative Aspects
Since its establishment, the Millennium Institute for Integrative Biology (iBio) has distinguished itself as a center of scientific excellence, underpinned by a robust and coherent institutional framework. At the core of iBio is a highly accomplished team of scientists, whose internationally recognized careers and complementary research lines enable a broad, integrative approach to the study of plant and fungal systems. This interdisciplinary synergy is a cornerstone of the Institute's ability to deliver cutting-edge scientific outcomes. Our labs are equipped with state-of-the-art technology, allowing for the design and execution of innovative and impactful experimental research. This technological capacity, coupled with the expertise of our multidisciplinary teams, supports scientific advancements that can turn into effective solutions in key sectors such as the agri-food industry — both within Chile and on the international stage. A defining feature of iBio is the strength of its international networks. From its inception, the Institute has cultivated and maintained strategic partnerships with leading research centers and academic institutions worldwide. These collaborations have significantly enhanced the quality of training for our students and early-career researchers, promoting the exchange of knowledge, access to advanced methodologies, and exposure to global scientific standards. Innovation is central to iBio's operational model. Through the convergence of diverse scientific disciplines and advanced research tools, the Institute has developed a dynamic problem-solving capacity that addresses real-world challenges, particularly in sectors critical to national development. This innovative capacity reinforces iBio's role as both a scientific and a socio-economic actor. Equally important is our strong commitment to science communication and public engagement. iBio maintains an active presence across various regions of the country, ensuring a continuous dialogue between the scientific community and society. These outreach initiatives enable the Institute to identify local challenges first-hand and co-develop solutions with direct and measurable impact on communities. The training of highly qualified human capital is a fundamental institutional priority. iBio provides its students and young researchers with comprehensive access to scientific resources, mentorship, and a collaborative research environment. Participation in international exchange programs further enriches their academic experience, offering exposure to diverse scientific cultures and practices.

Moreover, we encourage our trainees to participate in outreach and science communication activities, fostering a sense of social responsibility and reinforcing the broader relevance of their research efforts. This holistic training approach prepares a new generation of scientists equipped not only with technical expertise but also with a deep understanding of the societal impact of science.

Together, these institutional strengths firmly position iBio as a national and international research center.

Negative Aspects/Challenges

Since our inception in December 2017, we have pursued ambitious scientific goals with determination and passion. However, we have done so under increasingly difficult financial conditions. Our annual budget has remained unchanged since that first year, despite significant inflation and the rising costs of imported materials. And now it is further burdened by additional taxes on services which were previously exempted. Each year, this fixed budget loses value. According to the National Institute of Statistics (INE), the Consumer Price Index (CPI) rose by **44.5 %** between April 2018 and April 2025. In real terms, this has translated into a consistent decline in our purchasing power and, consequently, our ability to operate at full capacity.

Despite this reality, we have received no indication from the funding agency that the situation will change. On the contrary, recent announcements suggest new restrictions on funding eligibility, barring principal investigators from leading projects under other programs. For example, grants by the main program (FONDECYT), are individual and cannot be stacked (as with RO1s). Lately, increasing restrictions are limiting the type of grants in which a PI can participate, not considering that most of the program projects (like Institutes or centers) allocate a large fraction of the budget to human resources, empowering academic transitions of young scientists towards independence. Without increased financial support and with growing limitations on alternative funding sources, our ability to continue conducting world-class research in Chile is under serious threat. Our outputs — in publications, training, and innovation — will inevitably suffer.

Another important challenge is the uncertainty of iBio's future after completing the 10-year grant contract. The lack of a clear national policy regarding scientific centers, puts Institutes like ours in a complex scenario when it comes to projecting science into the future.

Likewise, there is little investment from the private sector in R&D which makes partnerships uncommon.

Another challenge we face is the **low number of researchers**. Chile ranks among the lowest in researcher density within the OECD. This is a structural weakness that can only be addressed by investing in scholarships and programs that train advanced human capital. Increasing the number of scientists is essential to propel innovation, solve national challenges, and generate knowledge that benefits all.

Finally, we must look to the roots: **science education**. Our educational system needs a deep and thoughtful review. Science should be accessible, engaging, and inspiring from an early age. This means investing in school libraries, equipping laboratories, and revising curricula to nurture curiosity and critical thinking in every child.

The path forward is challenging, but not impossible. What we need is a collective vision — one that recognizes science not as a luxury, but as a pillar of national development. Only then will we be able to build a resilient, innovative, and inclusive future for Chile.

10. Annexes:

Annex 1.- Research Lines

Nº	Research Line	Research Line Objectives	Description of Research Line	Researcher	Research Discipline
1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes	It seeks to understand how plants sense and respond to nitrogen nutrient signals, to identify GRN underlying light, time (circadian) and nutritional control of fungal physiology, to understand the role of phenotypic diversity on adaptation to cold environments.	To explore how environmental signals are perceived by plants or fungi, leading to transcriptional, metabolic and phenotypic changes..	LL,RG,EV,PC,FF,FC	Cell Biology Molecular Biology
2	To Assess The Effect of Environmental Signals on Interspecies Dynamics	We aim to understand molecular mechanisms integrating environmental and biotic signals	The second research line considers a more ambitious and realistic scenario where organisms face environmental changes in the presence of interacting beneficial or detrimental species.	RG,EV,FF,FC	Biotechnology Molecular Biology
3	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory	Implementation of orthogonal & tunable transcriptional switches permits controlled perturbation of transcriptional nodes of interest in plant or fungi, allowing to test hypotheses and models derived from both Aims 1 and 2. I	To develop new sets of synthetic controllers, processing light information (optogenetic switches) or molecular compounds (nitrate signal, quorum sensing like signals)	LL,RG,EV,PC	Biotechnology Molecular Biology
4	Implementation of open source technologies and promotion of open science	To create a platform of open source technologies for plant/fungal systems and synthetic biology	iBio is committed to having a long-lasting impact in education, capacity building and public engagement on biological sciences. Although this may seem an outreach or		Biotechnology Molecular Biology Cell Biology.

			training activity, we consider this to be a defining Aim of our Institute that will also substantially contribute to propel our research lines 1 to 3 forward		

Annex 2.- Institute / Nucleus Researchers

2.1 Summary Table – Center's Researchers

Category of researcher	Quantity	Average age	Nationality		Distribution Gender		
			National	International	Male	Female	Not stated
Director	1	51	1		1		
Alternate Director	1	53	1		1		
Principal Researcher	6	46	5	1	5	1	
Senior Researcher	8	44	6	2	6	2	
Young Researcher	12	40	11	1	6	6	
Postdoctoral	21	36	19	2	9	12	

2.2 Principal Researchers

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center
Luis Larrondo	RL1, RL3, RL4	Chilean	M	30/06/1973	Biochemistry	D	Pontificia Universidad Católica de Chile	Full Professor	2
Rodrigo Gutiérrez	RL1, RL2, RL3, RL4	Chilean	M	30-12-1971	Biochemistry	D	Pontificia Universidad Católica de Chile	Full Professor	2
Elena Vidal	RL1, RL2, RL3, RL4	Chilean	F	15-06-1979	Biochemistry	D	Universidad Mayor	Associate Professor	2
Paulo Canessa	RL1, RL3, RL4	Chilean	M	16-07-1979	Biochemistry	D	Universidad Andrés Bello	Associate Professor	2
Fernán Federici	RL1, RL2, RL4	Argentinian	M	20-11-1980	Biochemistry	D	Pontificia Universidad	Associate Professor	2

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center
							Católica de Chile		
Francisco Cubillos	RL1, RL2, RL4	Chilean	M	02-06-1983	Biotechnology	D	Universidad de Santiago de Chile	Associate Professor	2

2.3 Senior Researchers

Name	Research Line	Nationality	Gender	Date of birth dd/mm/yy	Profession	Academic Degree	Affiliation	Current Position	Relation with Center

NOMENCLATURE: **[Gender]** [M] Male [F] Female [ND] Does not Declare	**[Academic Degree]** [U] Undergraduate [M] Master [D] Doctoral	**[Relation with Center]** [1] Full time [2] Part time

Annex 3.- Publications (Total or partially financed by MSI)

3.1.- WOS Publications or Similar to WOS Standard:

3.1.1 Principal Researchers:

N°	Title	**Authors	DOI	Quartile*	Lines of Research	N° principal researchers of the center
1	An Open One-Step RT-qPCR for SARS-CoV-2 detection	Cerda, A., Rivera, M., Armijo, G., Ibarra-Henriquez, C., Reyes, J., Blázquez-Sánchez, P., Avilés, J., Arce, A., Seguel, A., Brown, A. J., Vásquez, Y., Cortez-San Martín, M., Cubillos, F. A., García, P., Ferres, M., Ramírez-Sarmiento, C. A., Federici, F., & Gutiérrez, R. A.	10.1371/journal.pone.0297081	Q1	Implementation of open source technologies and promotion of open science.	3
2	Phylogenetically diverse wild plant species use common biochemical strategies to thrive in the Atacama Desert	Dussarrat T, Nilo-Poyanco R, Moyano TC, Prigent S, Jeffers TL, Díaz FP, Decros G, Audi L, Sondervan VM, Shen B, Araus V, Rolin D, Shasha D, Coruzzi GM, Gibon Y, Latorre C,	10.1093/jxb/erae117	Q1	To Assess The Effect of Environmental Signals on Interspecies Dynamics.	1

		Pétriacq P, Gutiérrez RA				
3	Nitrogen sensing and regulatory networks: it's about time and space	Shanks, C. M., Rothkegel, K., Brooks, M. D., Cheng, C. Y., Alvarez, J. M., Ruffel, S., Krouk, G., Gutiérrez, R. A., & Coruzzi, G. M.	10.1093/plcell/koae038	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
4	Recent advances in local and systemic nitrate signaling in Arabidopsis thaliana	Delgado, L. D., Nunez-Pascual, V., Riveras, E., Ruffel, S., & Gutiérrez, R. A.	10.1016/j.pbi.2024.102605	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
5	Dynamic changes in mRNA nucleocytoplasmic localization in the nitrate response of Arabidopsis roots	Fonseca, A., Riveras, E., Moyano, T. C., Alvarez, J. M., Rosa, S., & Gutiérrez, R. A.	10.1111/pce.15018	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
6	The Characterization of a Novel PrMADS11 Transcription Factor from Pinus radiata Induced Early in Bent Pine Stem	Méndez, T., Guajardo, J., Cruz, N., Gutiérrez, R. A., Norambuena, L., Vega, A., Moya-León, M. A., & Herrera, R.	10.3390/ijms25137245	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
7	Genomes of the Orestias pupfish from the Andean Altiplano shed light on their evolutionary history and phylogenetic relationships within Cyprinodontiforms	Morales, P., Gajardo, F., Valdivieso, C., Valladares, M. A., Di Genova, A., Orellana, A., Gutiérrez, R. A., González, M., Montecino, M., Maass, A., Méndez, M. A.,	10.1186/s12864-024-10416-w	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1

		& Allende, M. L.				
8	Filling the gaps on root hair development under salt stress and phosphate starvation using current evidence coupled with a meta-analysis approach	Ibeas MA, Salinas-Grenet H, Johnson NR, Pérez-Díaz J, Vidal EA, Alvarez JM, Estevez JM.	10.1093/plphys/kiae346	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	1
9	Gene regulatory networks underlying sulfate deficiency responses in plants	Fernández JD, Miño I, Canales J, Vidal EA	10.1093/jxb/erae051	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
10	An integrative taxonomy approach reveals Saccharomyces chiloensis sp. nov. as a newly discovered species from Coastal Patagonia	Peña TA, Villarreal P, Agier N, De Chiara M, Barría T, Urbina K, Villarroel CA, Santos ARO, Rosa CA, Nespolo RF, Liti G, Fischer G, Cubillos FA.	10.1371/journal.pgen.1011396	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
11	Transcriptional rewiring of an evolutionarily conserved circadian clock	Goity A, Dovzhenok A, Lim S, Hong C, Loros J, Dunlap JC, Larrondo LF.	10.1038/s44318-024-00088-3	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	1
12	Wild Patagonian yeast improve the evolutionary potential of novel interspecific hybrid strains for lager brewing	Molinet J, Navarrete JP, Villarroel CA, Villarreal P, Sandoval FI, Nespolo RF, Stelkens R, Cubillos FA.	10.1371/journal.pgen.1011154	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
13	Domestication signatures in the non-conventional yeast Lachancea cidri	Pablo Villarreal, Samuel O'Donnell, Nicolas Agier,	10.1128/msystems.01058-23open	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1

		Felipe Muñoz-Guzman, Jose Benavides-Parra, Kami Urbina, Tomas A. Peña, Mark Solomon, Roberto F. Nespolo, Gilles Fischer, Cristian Varela, Francisco A. Cubillos				
14	Mg-Al LDH nanosheets as a nanotechnological tool in agriculture: An exploratory toxicity evaluation study.	Salinas-Jiménez R, Vera G, Tobar M, Moscote J, Acha G, Herrera-Vásquez A, Rojas-Rivera D, Vidal EA, Miyasaka Almeida A, Ahumada M	10.1039/D3EN00733B	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
15	Climate change and population persistence in a hibernating marsupial	Nespolo RF, Quintero-Galvis JF, Fontúrbel FE, Cubillos FA, Vianna J, Moreno-Meynard P, Rezende EL, Bozinovic F	10.6084/m9.figshare.c.7262689.	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	1
16	Nakazawaea atacamensis f.a., sp. nov. a novel nonconventional fermentative ascomycetous yeast species from the Atacama Desert	Araya M, Villarreal P, Moyano T, Santos ARO, Díaz FP, Bustos-Jarufe A, Urbina K, Del Pino JE, Groenewald M, Gutiérrez RA, Rosa CA, Cubillos FA.	10.1002/yea.3920	Q2	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	2
17	Site-Specific Nutrient Data	Hua X, Lusk CH, Dickie IA,	10.1111/geb.13936	Q1	Unraveling Molecular Mechanisms of Organismal	1

	Reveal the Importance of Soils in Driving the Mycorrhizal Make-Up of Woody Vegetation Worldwide	Adu-Bredu S, Allen KJ, Araus V, Augusto L, Barsukov P, Bauman D, Brédoire F, Burslem D F. R. P., Dalling JW, Depauw L, Dexter KG., Drouet T, Godlee JL, Godoy R, Gutiérrez RA., Ilunga Muledi J, Jacobs A, Kooyman R, Latorre C, López Angulo J, Macé S, Maes SL, Gonçalves FMP, Marimon Junior BH, Nicolas M, Nilus R, O'Brien M, Oliveras Menor I, Piper FI, Read J, Reynolds G, Saldaña A, Schwantes Marimon B, Verheyen K, Westoby M, Wigley B, Wright IJ			Responses to Environmental Changes.	

Q4: Ingresar esta opción para aquellos artículos que no posean cuartil.
Ingresar solamente los apellidos de investigadores del centro, para el resto de los autores, utilizar la expresión "et al." para indicar que hay más autores

3.2.- SCOPUS Publications or Similar to SCOPUS Standard:

3.2.1 Principal Researchers:

N°	Title	**Authors	DOI	Quartile*	Lines of Research	N° principal researchers of the center
1						
2						
3						

3.3.- SCIELO Publications or Similar to SCIELO Standard

3.3.1 Principal Researchers:

N°	Title	**Authors	DOI	Quartile*	Lines of Research	N° principal researchers of the center
1						
2						
3						

3.4.- Scientific Books and Chapters

3.4.1 Principals Researchers:

N°	Title	Autor	URL/Link	ISBN	Lines of Research	N° principal researchers of the center
1						
2						
3						

3.5.- Other Publications

3.5.1 ***Principals Researchers****:*

N°	Title	Publication Category	Other Category	Authors	DOI	Quartile	Lines of Research	N° principal researchers of the center
1								
2								
3								

3.6.- Other researchers: "WOS Publications or Similar to WOS Standard", "SCOPUS Publications or Similar to SCOPUS Standard" "SCIELO Publications or Similar to SCIELO Standard", "Books and chapters in books" y "Other Publications [Other Researchers]":

N°	Title	Publication Category	Other Category	Authors	DOI/URL	Quartile	Lines of Research	N° principal researchers of the center
1	Auxin signaling gets oxidative to promote root hair growth	ISI/WOS o Similar a ISI/WOS standard		Gabarain VB, Ibeas MA, Salinas-Grenet H, Estévez JM	10.1016/j.molp.2024.04.007	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
2	Exploring the puzzle of reactive oxygen species acting on root hair cells	ISI/WOS o Similar a ISI/WOS standard		López LE, Ibeas MA, Diaz Dominguez G, Estevez JM	10.1093/jxb/erae260	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
3	Arabidopsis pollen	ISI/WOS o Similar a		Sede AR, Wengier DL, Borassi	10.1093/jxb/erae269	Q1	Unraveling Molecular Mechanisms of	

	prolyl-hydroxylases P4H4/6 are relevant for correct hydroxylation and secretion of LRX11 in pollen tubes	ISI/WOS standard		C, Ricardi M, Somoza SC, Aguiló R, Estevez JM, Muschietti JP.			Organismal Responses to Environmental Changes.	
4	New molecular components that regulate the transcriptional hub in root hairs: coupling environmental signals with endogenous hormones to coordinate growth	ISI/WOS o Similar a ISI/WOS standard		Lopez LE, Chuah YS, Encina F, Carignani Sardoy M, Berdion Gabarain V, Mutwil M, Estevez JM	10.1093/jxb/erad419	Q1	To Assess The Effect of Environmental Signals on Interspecies Dynamics.	
5	RALF22 is a key modulator of the root hair growth responses to fungal ethylene	ISI/WOS o Similar a ISI/WOS standard		León Morcillo RJ, Leal-López J, Férez-Gómez A, López-Serrano L, Baroja-Fern	10.1093/plphys/kiae484	Q1	To Assess The Effect of Environmental Signals on Interspecies Dynamics.	

	emissions in Arabidopsis			ández E, Gámez-Arcas S, Tortosa G, López LE, Estevez JM, Doblas VG, Frías-España L, García-Pedrajas MD, Sarmiento-Villamil J, Pozueta-Romero J				
6	Transcription factor NAC1 activates expression of peptidase-encoding AtCEPs in roots to limit root hair growth	ISI/WOS o Similar a ISI/WOS standard		Rodríguez-García, D. R., Rondón Guerrero, Y. D. C., Ferrero, L., Rossi, A. H., Miglietta, E. A., Aptekmann, A. A., Marzol, E., Martínez Pacheco, J., Carignani, M., Berdion Gabarain, V., Lopez, L. E., Díaz Dominguez, G., Borassi, C., Sánchez-Serrano, J. J., Xu, L., Nadra, A. D., Rojo, E., Ariel, F., & Estévez, J. M.	10.1093/plphys/kiad533	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	

7	Fusarium sp. Strain K-23 Alleviates Salt Stress in Arabidopsis thaliana Through its Root Hair Growth-Promoting Effect	ISI/WOS o Similar a ISI/WOS standard		Francis C Onejeme, Adrián González Ortega-Villaizán, Estefanía Rodríguez-Dobreva, Basha Topel Prieto, Manish K Patel, Selma Guendouzi, Priya YN Reddy, Leonel E Lopez, José M Estevez, Karaba N Nataraja, R Uma Shaanker, Begoña Benito, Jesús Vicente-Carbajosa, Ralf Oelmüller, Stephan Pollmann	10.1007/s00344-024-11518-1	Q1	To Assess The Effect of Environmental Signals on Interspecies Dynamics.	
8	The exception to the rule? TORC1 triggers growth under low nutrient environments	ISI/WOS o Similar a ISI/WOS standard		López DE, Pacheco JM, Estévez JM.	10.1016/j.tplants.2023.10.001	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	
9	DNA controls the dimerization of	ISI/WOS o Similar a ISI/WOS standard		Kolimi N, Ballard J, Peulen T, Goutam R, Duffy FX	10.1016/j.xcrp.2024.101854	Q1	Implementation of open source technologies and promotion of open science.	

	the human FoxP1 forkhead domain			3rd, Ramírez-Sarmiento CA, Babul J, Medina E, Sanabria H.				
10	Concerted transformation of a hyper-paused transcription complex and its reinforcing protein	ISI/WOS o Similar a ISI/WOS standard		Zuber PK, Said N, Hilal T, Wang B, Loll B, González-Higueras J, Ramírez-Sarmiento CA, Belogurov GA, Artsimovitch I, Wahl MC, Knauer SH	10.1038/s41467-024-47368-4	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
11	Dissecting the structural and functional consequences of the evolutionary proline-glycine deletion in the wing 1 region of the forkhead domain of human FoxP1	ISI/WOS o Similar a ISI/WOS standard		Tamarín S, Galaz-Davison P, Ramírez-Sarmiento CA, Babul J, Medina E	10.1002/1873-3468.14972	Q3	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
12	Current challenges for	ISI/WOS o Similar a		Auge G, Sunil RS, Ingle RA,	10.1111/nph.20083	Q1	Implementation of open source technologies and	

	plant biology research in the Global South	ISI/WOS standard		Rahul PV, Mutwil M, Estevez JM			promotion of open science.	
13	The trade-off between grain weight and grain number in wheat is explained by the overlapping of the key phases determining these major yield components	ISI/WOS o Similar a ISI/WOS standard		Vicentin L, Canales J, Calderini DF	10.3389/fpls.2024.1380429	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	
14	Metamorphic proteins and how to find them	ISI/WOS o Similar a ISI/WOS standard		Porter LL, Artsimovitch I, Ramírez-Sarmiento CA	10.1016/j.sbi.2024.102807.	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
15	The Light Chain Allosterically Enhances the Protease Activity of Murine Urokinase-Type Plasmin	ISI/WOS o Similar a ISI/WOS standard		Torres-Paris C, Song HJ, Engelberger F, Ramírez-Sarmiento CA, Komives EA	10.1021/acs.biochem.4c00071	Q3	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	

	ogen Activator							
16	A contact-based analysis of local energetic frustration dynamics identifies key residues enabling RfaH fold-switch	ISI/WOS o Similar a ISI/WOS standard		González-Higueras J, Freiberger MI, Galaz-Davison P, Parra RG, Ramírez-Sarmiento CA	10.1002/pro.5182ope	Q1	Implementation of open source technologies and promotion of open science.	
17	Geomicrobiological characterization of the evaporitic ecosystem in the hypersaline lake Laguna Verde (Andean Puna, Northwestern Argentina)	ISI/WOS o Similar a ISI/WOS standard		Saona LA, Villafañe PG, Carrizo D., Cónsole Gonella C., Néspolo R.,. Farías ME.	10.1002/ece3.10931	Q1	To Assess The Effect of Environmental Signals on Interspecies Dynamics.	
18	Proteomic profiling of Arabidopsis nuclei reveals distinct	ISI/WOS o Similar a ISI/WOS standard		Muñoz-Díaz E, Fuenzalida-Valdivia I, Darrière T, de Bures A, Blanco-Herrera F, Rompais	10.1038/s41598-024-65558-4.	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	

	protein accumulation kinetics upon heat stress			M, Carapito C, Sáez-Vásquez J.				
19	The art of modeling gene regulatory circuits	ISI/WOS o Similar a ISI/WOS standard		Gómez-Schiavon M, Montejano-Montelongo I, Orozco-Ruiz FS, Sotomayor-Vivas C.	10.1038/s41540-024-00380-2	Q2	Implementation of open source technologies and promotion of open science.	
20	Extrinsic fluctuations in the p53 cycle	ISI/WOS o Similar a ISI/WOS standard		Hernández-García ME, Gómez-Schiavon M, Velázquez-Castro J.	10.1063/5.0227728.	Q1	Implementation of open source technologies and promotion of open science.	
21	Phenotyping of a new yeast mapping population reveals differences in the activation of the TORC1 signalling pathway between wild and domesticated yeast strains	ISI/WOS o Similar a ISI/WOS standard		Rocha G., Gómez M., Baeza C., Salinas F., Martínez C., Kessi-Pérez E.	10.1186/s40659-024-00563-5	Q1	Design and Development of Molecular Loops and Analyses of Transcriptional Environmental Memory.	
22	Local adaptation of Dromicops	ISI/WOS o Similar a ISI/WOS standard		Quintero-Galvis JF, Saenz-Agudelo P, D'Elía G,	10.1002/ece3.70355	Q1	Unraveling Molecular Mechanisms of Organismal Responses to	

	marsupials (Microbiotheriidae) from southern South America: Implications for species management facing climate change			Nespolo RF.			Environmental Changes.	
23	The root hairless mutant buzz in Brachypodium distachyon shows increased nitrate uptake and signaling but does not affect overall nitrogen use efficiency	ISI/WOS o Similar a ISI/WOS standard		Rosas MA, Álvarez JM, Sanguinet KA.	10.1111/tpj.17143	Q1	Unraveling Molecular Mechanisms of Organismal Responses to Environmental Changes.	

3.7.- Collaborative publications:

Category of Publication	1 researcher		2 researchers		3 researchers		4 or more researchers	
	Nº	%	Nº	%	Nº	%	Nº	%
WOS Publications or Similar to WOS Standard	22	53,66%	7	17,07%	6	14,63%	6	14,63%

SCOPUS Publications or Similar to SCOPUS Standard	0	0,00%	0	0,00%	0	0,00%	0	0,00%
SCIELO Publications or Similar to SCIELO Standard	0	0,00%	0	0,00%	0	0,00%	0	0,00%
Books and chapters	0	0,00%	0	0,00%	0	0,00%	0	0,00%
Other Publications	0	0,00%	0	0,00%	0	0,00%	0	0,00%
Total of publications	**22**	**53,66%**	**7**	**17,07%**	**6**	**14,63%**	**6**	**14,63%**

Annex 4.- Education and capacity building

Annex 4.- Short-term Traineeships of MSI students

4.1 List of Internships

Student name	Institution	Country	Advisor	Project Description	Starting Date [dd/mm/yy]	Ending Date [dd/mm/yy]
Pablo Quintrel Poblete	Sorbonne Université	Francia	Gilles Fisher	Phenotyping experiments, QTL analysis, and validation of genes involved in maltose consumption	19-12-23	19-03-24
Christian Ignacio Oporto Donoso	Academia Sinica	Taiwan	Isheng Jason Tsai	Análisis filogenético y estructura poblacional de aislados naturales de S. euabayanus	29-01-24	27-04-24
Agustín Aníbal Cofré Sandoval	Université Côte d'Azur	Francia	Gianni Liti	Use of quantitative trait analysis in intraspecific hybrids of *S. eubayanus* for fermentative phenotypes	01-06-24	28-08-24
Valentina Andrea Núñez Pascual	Sainsbury Laboratory of Cambridge University	Inglaterra	Dra. Sarah Robinson	Measurements of cell wall biomechanical	07-03-24	14-06-24

				properties using AFM and ACME		
Jose David Fernandez Perez	Instituto de Biología Integrativa de Sistemas I2SysBio, U. de Valencia	España	Elena Vidal	IDENTIFICATION OF GENE REGULATORY NETWORKS CONTROLLING SULFATE DEFICIENCY RESPONSE IN SOLANUM LYCOPERSICUM	18-02-24	27-04-24
Diego Felipe Landaeta Sepulveda	Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, INIA-CSIC	España	Elena Vidal	Gene regulatory networks controlling root system architecture under different Nitrogen and water availability conditions in Solanum lycopersicum	14-10-24	10-12-24
Cyndi Andrea Tabilo Agurto	The Ohio State University	Estados Unidos de América	Irina Artsimovitch	Characterization of RfaH variants through in vitro transcription and in vivo translation	02-10-24	29-12-24
Aransa Fernanda Griñen Muñoz	AI Proteins Inc.	Estados Unidos de América	Christopher Bahl, Ben Meinen	De novo generation of PET-degrading enzymes using Artificial Intelligence	28-06-24	
José Ignacio Costa Cialdella	University of California - Santa Cruz	Estados Unidos de América	Luis Larrondo / Carrie Partch	Biophysical analyses aimed at evaluating the auto-inhibitory role of CK1 tail	01-01-24	20-09-24

4.2 List of External Internships

Intern Type	Intern Name	Academic Degree	Home Institution	Destination Institution	Country	Project Description	Starting Date [dd/mm/yy]	Ending Date [dd/mm/yy]
Student	Sergio Izquierdo	PhD	Universidad Complutense de Madrid	USACH	Spain	Genome analysis of *Yarrowia lipolytica*	01-04-24	30-06-24
Student	Nina Vittorelli	PhD	Universidad de Sorbonne	USACH	France	Experiments to measure the response to alpha factor and mating behavior in native yeast strains	04-11-24	12-12-24
Student	Adrian González	PhD	Centro de Biotecnología y Genómica de la plantas (CBGP)	PUC	Spain	Evaluation of experiments through bioinformatic analysis	31-05-24	30-08-24
Student	Macarena Mellado	PhD	Instituto de Biología Molecular y Celular de Plantas CSIC-UPV	PUC	Spain	Experiments on *Marchantia polymorpha* strains	23-09-24	23-11-24
Student	Maria Fernanda Mendoza	PhD	Universidad de Guanajuato	PUC	México	To work on cell free reactions	09-09-24	31-10-24
Investigator	Matías Villaroel	PhD	Instituto Leloir	PUC	Argentina	To work on DNA assembly methods	01-03-24	30-05-24

Student	Kavi Shah	PhD	University of Cambridge	PUC	England	To work on interviews about the biotech landscape in Latam	22-04-24	31-05-24
Investigator	Marc Dusseiller	Doctorado	Hackteria	PUC	Sweden	To explore art-science projects	22-04-24	13-06-24
Student	Gabriel Couillaund	Magister	Wageningen University	UC-UNAB	Holland	Proteomic analyses of Botrytis cinerea circadian system	01-04-24	01-10-24
Student	Amelie Mallevielle	Magister	Universidad de Montpellier	PUC	France	Experiments in Hoffmannseggia doelli	11-03-24	28-03-24

Annex 5- Networking and other collaborative work

5.1. Formal Collaboration networks

Network Name	Network Scope	Description	Researchers				Institutions
			From the Center		External		
			Researchers	Postdocs/ Students	Researchers	Postdocs/ Students	
CENIA-iBio	I	Cooperation and synergistic work for development and support in the research field using artificial intelligence in biological sciences, framed within our RL4. This objective proposes the use of natural language processors to mine scientific literature in order to identify new testable experimental ideas.	1	1	5	2	CENIA iBio
Resilomics Net project FOVI230159	I	Establish a partnership with leading Spanish research institutions to strengthen high-impact scientific and technological research, as well as to train highly qualified human capital in advanced technologies in Genomics, Systems Biology, and Biotechnology. This effort aims to improve the	2	4	7	6	UNAB U Austral USACH U O´Higgins U de Valencia U Autónoma de Barcelona

		productivity of agriculturally and aquaculturally important species of national relevance, considering the challenges posed by climate change					
Millennium Nucleus in Data Science for Plant resilience Phytolearning	N	ANID-Millennium Science Initiative Program - NCN2024_047	3	1	5	0	UNAB U Mayor U Adolfo Ibañez U de Temuco U de O´Higgins U de Concepción
Anillo ACT210007	N	ANID-Anillo ACT210007	1	0	4	4	U Mayor INIA
ECOS230001	I	Unravelling the role of Structural Variants on the reproductive and physiological divergence between Patagonian and Holarctic populations	2	3	3	2	USACH Sorbonne Université
National Science Foundation (NSF)	I	Two resources critical to plant growth, Nitrogen (N) and Water (W), are limited in soils globally, creating marginal soils that are agriculturally unproductive. To engineer or	1	3	4	4	NYU UNAB

		breed crop varieties that can thrive in marginal environments, it must first be understood how plants sense and integrate responses to Nitrogen (N) and Water (W). Detecting the genes and gene regulatory mechanisms plants rely on to sense and respond to multiple environmental inputs is at the leading edge of efforts to adapt crops to a changing climate. Thus, this award resides in Pasteur's quadrant, the scientific space where fundamental scientific discoveries have applied outcomes.					

NOMENCLATURE:
[Network Scope]
[N] National [I] International [LA] Latin American

Annex 5.2.- Collaboration Networks

Activity Name	Objective	Description	Co-Participants Institutions	Product	Number of Researchers from the Center	Number of Students from the Center
Collaborative internship	Single-cell RNA sequencing of cotyledons grown under contrasting conditions using Parse Biosciences technology	Single-cell RNA sequencing of cotyledons grown under contrasting conditions using Parse Biosciences technology.	Dr. Malcolm Bennett, University of Nottingham	Publication	2	0
Novel global regulators of differentiation and pathogenicity as tools for RNAi targeted control of fungal plant pathogens	Identification of transcription factors associated with infection in *Botrytis cinerea*.	Identification of transcription factors associated with infection in *Botrytis cinerea*.	Universidad de Salamanca	Proyecto de investigación	2	1
ResilomicsNet project FOVI230159	Establish a partnership with leading Spanish research institutions to strengthen high-impact scientific and technological research, as well as the training of highly qualified human capital in	Resilomics Net: Establishment of an international partnership to enhance the climate resilience of species with productive value	Universidad Andrés Bello, Universidad Austral, Universidad de Santiago de Chile, Universidad de O'higgins, Universidad de Valencia, Universidad Autónoma de Barcelona	Publication	2	2

	the latest technologies in Genomics, Systems Biology, and Biotechnology. This collaboration aims to improve the productivity of agriculturally and aquaculturally important species of national relevance in the context of climate change.					

Annex 6. - Outreach

6.1. Outreach activities throughout the period

Event Title	Type of Event	Target Audience	Región	Description Activity	N° of Student from the Center	Participating Researchers
Plants under environmental stress: overcoming current climate challenges.	Workshop	Professionals from the center's area of expertise		Workshop aimed at analyzing strategies to harness photosynthetic organisms in addressing the climate crisis, an unprecedented global challenge for humanity.	2	José Manuel Estévez Rodrigo Gutiérrez
Workshop: The Living Rhizosphere	Museum exhibition	General public	Región Metropolitana de Santiago	The initiative aims to raise awareness about the effects of climate change and highlight the role of science in finding solutions, such as developing plants with enhanced nutrient absorption and improved immunity	2	María Francisca Blanco Herrera. José Manuel Estévez Lopez
Open-source technologies for secondary education	Workshop	High school teachers.	Región de Magallanes y Antártica Chilena	High school teachers use open-source hardware and bacteria to perform low-cost PCR reactions. Bacteria that produce polymerases were used, allowing direct utilization without any purification.	3	Fernán Federici Noe
Open Tools for research	Workshop	General public		https://federicilab.org/2024/10/29/taller-de-tecnologias-abiertas-open-hardware-cell-free-open-enzymes-en-uncuyo-mendoza-junto-a-hackteria/	5	Fernán Federici Noe

Lecture and activity at Liceo Bicentenario Hualañé for senior high school students.	Interactive Worksh op	High School students	Región del Maule	Lecture and activity at Liceo Bicentenario Hualañé for senior high school students.	1	Ariel Cerda Rojas. José Miguel Alvarez Herrera.

Annex 6.2- Organization of Scientific Events

Scope	Title	Type of Event	City	Country	Responsible Researcher
National	"How the environment causes genetic changes in plants" Dr. Ralph Bock	Seminar	Santiago	Chile	Luis Larrondo Castro
National	Summer School of Mathematics – Biomathematics Workshop	Workshop	Querétaro	México	Mariana Gómez SChiavon
National	Systems Biology & Control Retreat	Conference	Querétaro	México	Mariana Gómez SChiavon
National	Workshop Single-Cell	Workshop	Santiago	Chile	Jose Miguel Alvarez Herrera
National	Plant System Biology: from molecules to the ecosystem, Simposio SBBM	Simposium	La Serena	Chile	Rodrigo Gutiérrez Ilabaca
National	iBio International Seminar with Dr. Ken Wolfe and Dr. Geraldine Butler	Seminar	Santiago	Chile	Francisco Cubillos Riffo
National	Seminar on plant gene regulation and functional genomics	Seminar	Santiago	Chile	Rodrigo Gutiérrez Ilabaca
National	Advanced Course in Molecular Biology and Plant Biotechnology	Other	Santiago	Chile	Jose Manuel Estevez Lopez
International	8th International Conference on Plant Cell Wall Biology	Other	Santiago	Chile	Jose Manuel Estevez Lopez
International	Plants under environmental stress: overcoming current climate challenges	Workshop	Baeza	España	Rodrigo Gutiérrez Ilabaca

National	"R Doesn't Bite" Course	Workshop	SANTIAGO	Chile	Jose Miguel Alvarez Herrera
National	Predicting and designing proteins of the future using Rosetta and Artificial Intelligence	Workshop	Santiago	Chile	Cesar Antonio Ramirez Sarmiento

6.3.- Articles and Interviews

Type of media and scope	Local/Regional		National		International		
	N° Interviews	N° Articles	N° Interviews	N° Articles	N° Interviews	N° Articles	TOTAL
Written	0	57	0	31	0	0	**88**
Internet	0	7	14	102	0	1	**124**
Audiovisual	0	0	20	1	0	0	**21**
TOTAL	**0**	**64**	**34**	**134**	**0**	**1**	**233**

6.4 Products of Projection to the External Environment (PME) (OPTIONAL)

Nº	Date	Product	Objective	Researchers	Target audience	Type	Scope

Annex 7. - Connections with other sectors:

Activity	Type of Activity [Number]	Institution Country	Agent Type [Number]	Economic Sector
Determination of antifungal activity	1	USA	1	Agriculture
Large-scale Lager beer production (550 L) using native yeast hybrids	2	Chile	1	Beer Industry

Large-scale Lager beer production (550 L) using native yeast hybrids	2	Chile	1	Beer Industry
Recovery of drought- and low-nitrogen-tolerant desert potato: Establishment of a cultivation method and marketing strategy for its commercialization	2	Chile	2	Agriculture
Experimental beer production using lager hybrids (50 L)	4	Chile	1	Beer Industry

NOMENCLATURE:

[Type of Activity] [1] Development of Studies [2] Project Implementation [3] Training [4] Prospective Activity [5] Scientific Training [6] Installation of Scientists [7] Others (specify at the table foot other types of activity)

[Agent Type] [1] Industry and Services [2] Organizations and Public Services [3] Educational Sector

Annex 8.- Relevant materials, products and activities carried out by the center (Máximo 3 Páginas) OPTIONAL:

Cellular reagents used in PME activity to allow high school teachers to produce their own enzyme in *E. coli* and use them directly in PCR reactions, avoiding costly and cumbersome protein purification steps. This work was achieved thanks to the use of open hardware. (Work in collaboration with Jenny Molloy).

Annex 9.- Figures

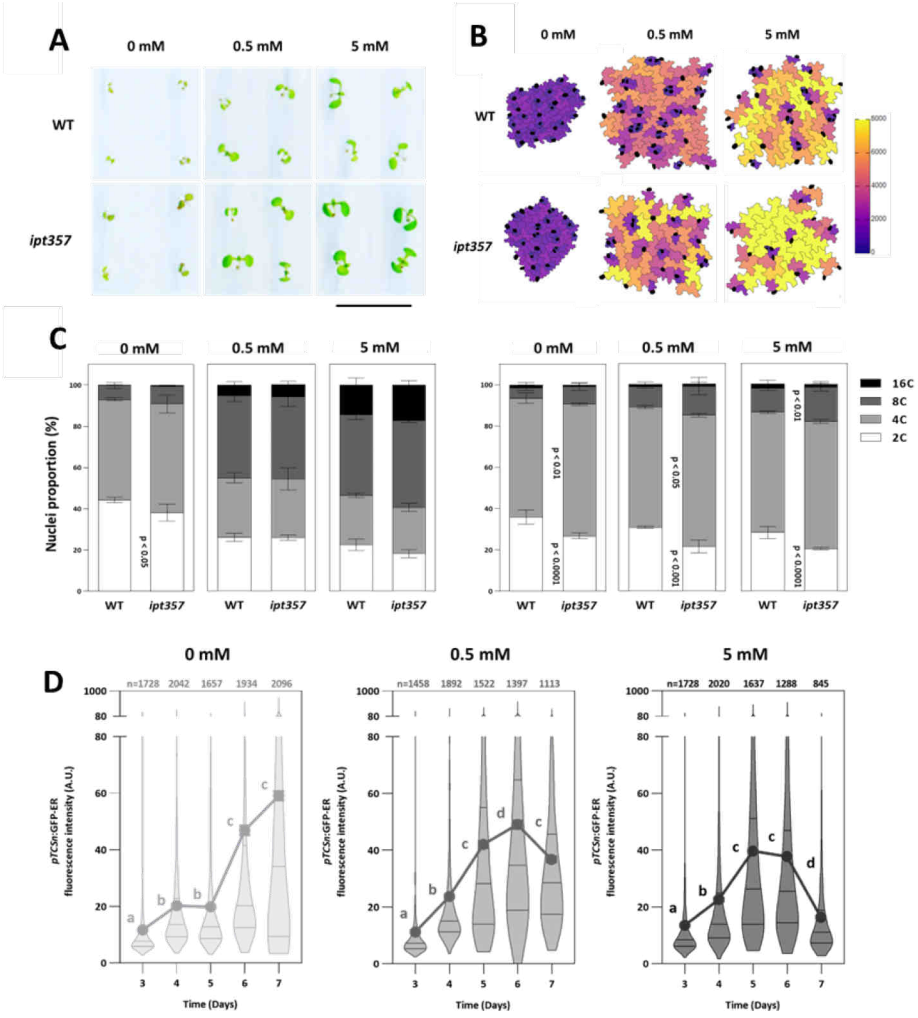


Figure 1. Nitrate interacts with cytokinin to modulate cotyledon growth. A) The growth promoting effect of nitrate is enhanced in 7-day-old CK-biosynthesis defective *ipt357* mutants compared to wild-type (WT) seedlings, indicating that CK restricts cotyledon growth in response to nitrate. **B**) Cell expansion is enhanced in the *ipt357* mutant compared to WT seedlings in response to higher nitrate concentrations, showing that CK limits cell expansion in response to nitrate. The color scale represent cell areas (μm^2). **C**) There are no statistically significant differences in ploidy levels in 7-day-old *ipt357* mutants compared to WT seedlings in response to higher nitrate concentrations (left). Ploidy levels are increased in 4-day-old *ipt357* mutants compared to WT seedlings in response to higher nitrate concentrations (right), elucidating that CK delays the endoreplication onset. Statistically significant differences were calculated using two-way ANOVA at each nitrate concentration. The proportion of nuclei per ploidy level were compared between genotypes using Bonferroni post test. **D**) The fluorescent signal of the p*TCS*n:GFP reporter line was assessed during cotyledon development upon different nitrate regimes. The p*TCS*n is a synthetic promoter that reports active CK signaling. We observed that nitrate accelerates the p*TCS*n:GFP fluorescent signal upregulation and enhances its downregulation. Therefore, nitrate modulates the pace of CK signaling activation and the extent of its downregulation during cotyledon development. Statistical differences were calculated using the Kruskal-Wallis test. (A-D) Altogether, our data indicates that early during post-germinative growth, nitrate-induced CK signaling favors the cell cycle and inhibits endoreplication. In turn, nitrate favors endoreplication, cell expansion and cotyledon growth by downregulating CK signaling later during post-germinative growth. Therefore, nitrate modulates CK signaling to modulate the cell cycle and ultimately cotyledon growth.

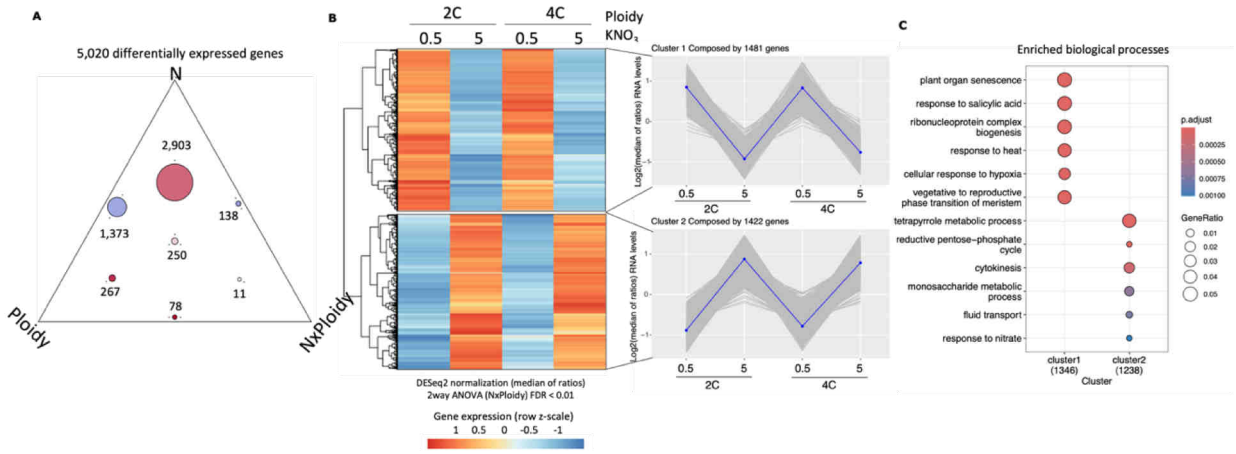


Figure 2. Nitrate is the main factor modulating the transcriptome in *Arabidopsis thaliana* shoots. A) Sungear plot showing the number of regulated genes by nitrate (N), ploidy and the interaction between nitrate and ploidy (NxPloidy) after a 2way ANOVA (FDR < 0.01) analysis. Circles located at vertex are genes regulated only by one condition, while circles between vertex are shared between two conditions and the circle located in the middle are genes shared by all conditions. Each circle represents the intersection between gene lists and their color indicates higher (red)- or lower (blue) significance. **B**) RNA levels and clustering of 2,903 genes regulated by 0.5- or 5-mM nitrate (KNO_3). Gene expression is shown for 2C and 4C sorted nuclei. **C**) Gene ontology (GO) terms showing significantly (q-value < 0.05) overrepresented biological processes from genes of cluster 1 and 2. Circle size indicates the number of genes belonging to that process and color shows significance.

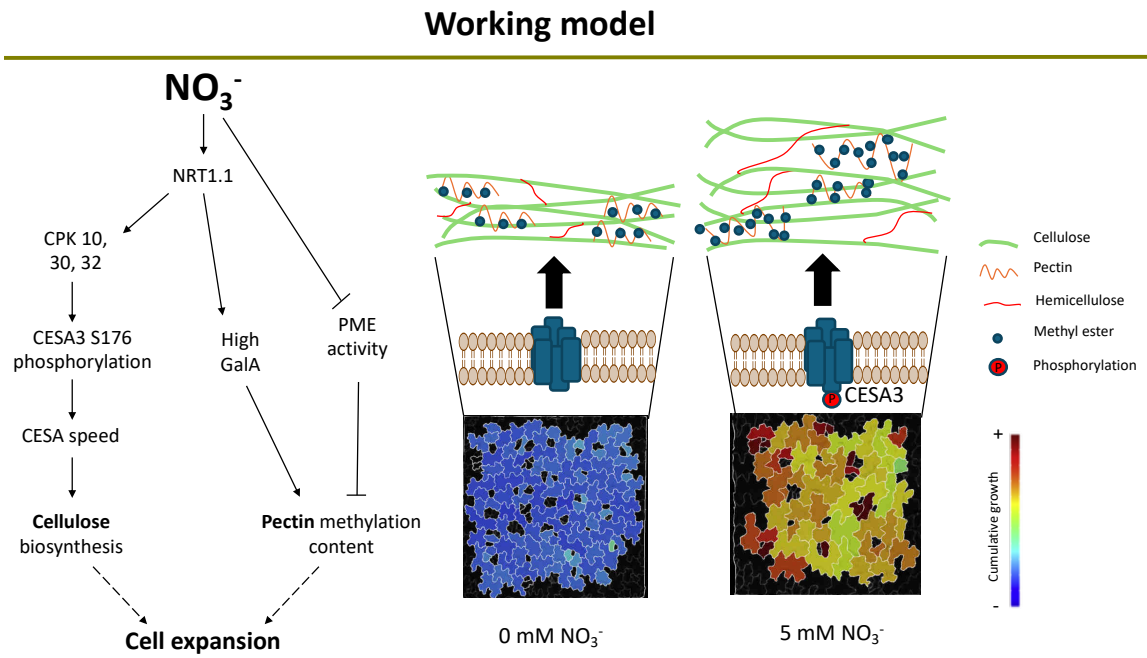


Figure 3. Working model of cell expansion under nitrate conditions. We have advanced our working model in which nitrate regulates the phosphorylation of CESA3 in S176 residues. The phosphorylation of CESA, by CPK10, 30, 32, has an impact in CESA speed and cellulose biosynthesis during cell expansión mediated by nitrate. In addition, nitrate regulates the GalA levles and decrease the PME activity. Both process showed a increase of pectin methylation content.

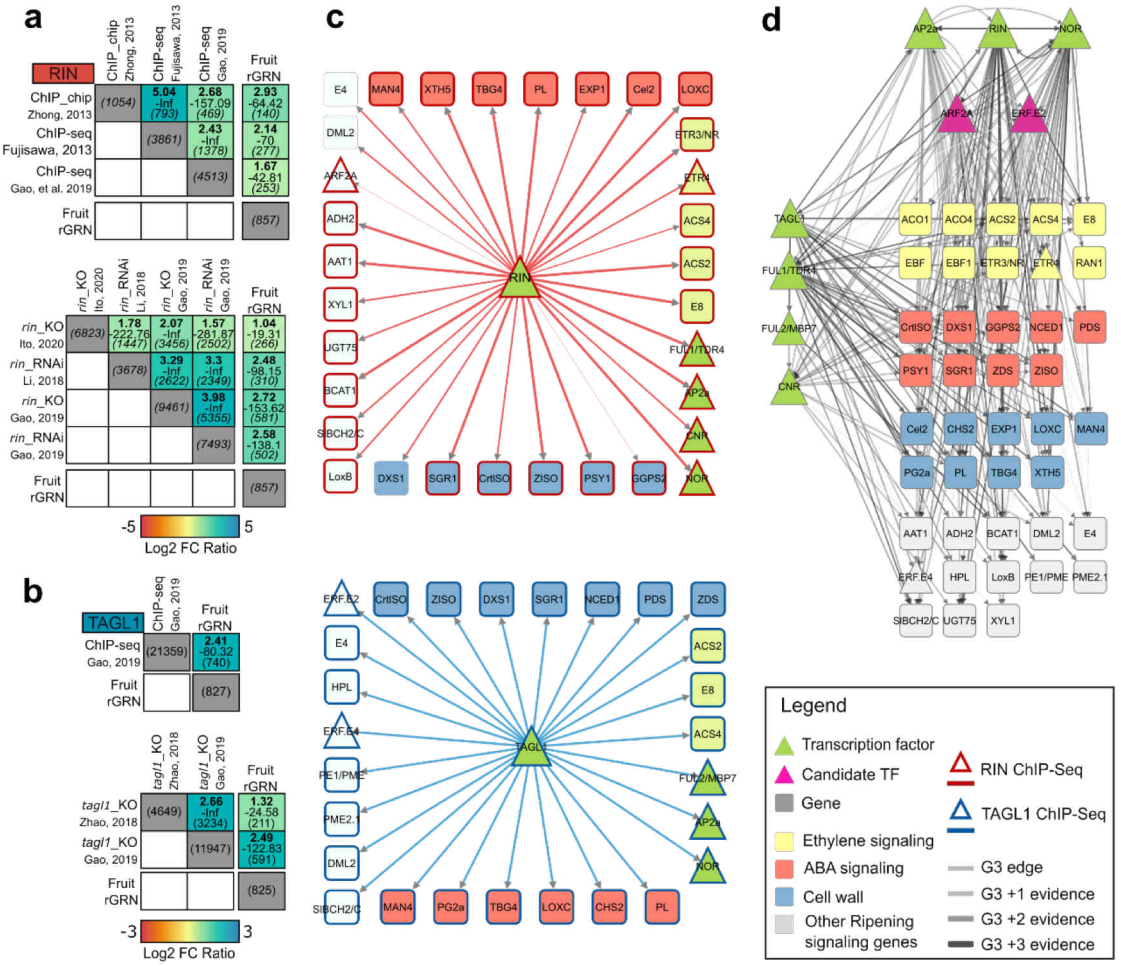


Figure 4. Tomato GRNs identify key TFs regulating fruit ripening-responsive genes. A-B. Enrichment and validation of fruit-specific GRNs for RIN and TAGL1 against gene sets from knockout mutants and ChIP analyses. Heatmap colors and first-row values indicate enrichment magnitude, the second row shows significance (adjusted p-value), and the third row displays the intersection size. **A**) Enrichment analysis for RIN targets. **B**) Enrichment analysis for TAGL1 targets. **C**) Networks of ripening-responsive genes showing RIN (top) and TAGL1 (bottom) GRNs. Node colors denote gene functions, node borders and edge colors indicate ChIP-seq binding evidence. **D**) Network visualization of the key regulators of ripening-responsive genes, with candidate top regulators highlighted as violet nodes.

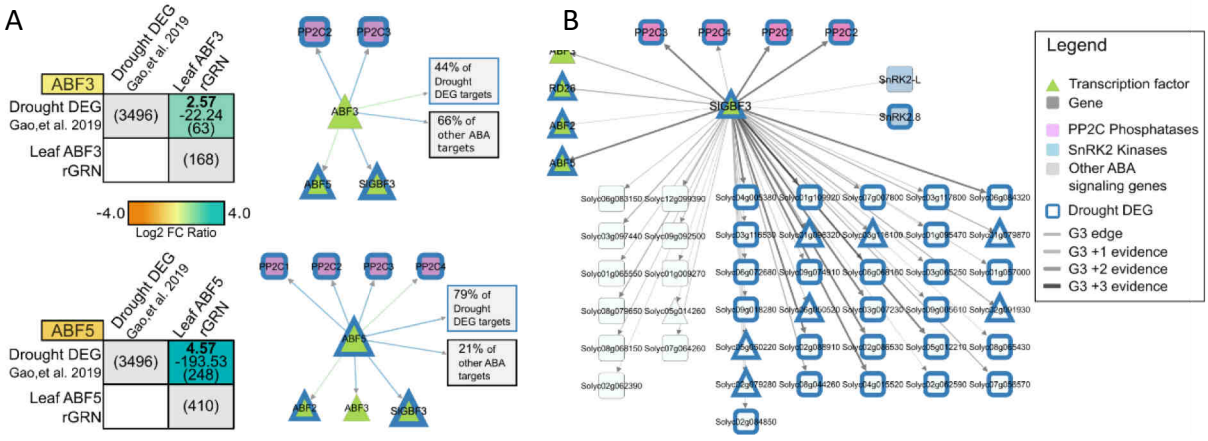


Figure 5. Tomato organ-specific GRNs point out new regulators of the Abscisic acid (ABA) responsive genes. **A**) Enrichment and validation of leaf-specific GRNs for ABF3 and ABF5 a list of DEGs from leaves during drought stress treatment, visualized as box heatmaps (left). Heatmap colors and first-row values indicate enrichment magnitude, the second row shows significance (p-value), and the third row displays the intersection size. Networks representing the main ABA responsive targets and drought regulatory targets (right) **B**) Network visualization of the ABA responsive genes regulated by *Sl*GBF3 within the leaf specific GRNs.

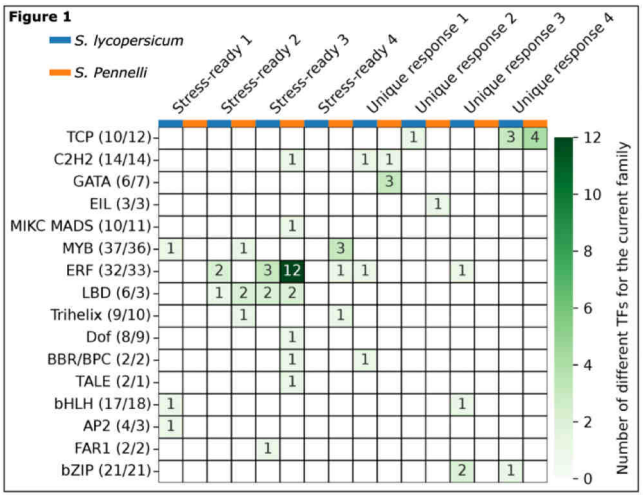


Figure 6. Promoter divergence of between *S. lycopersicum* and *S. pennellii*. Divergence in TF family enrichment between *S. lycopersicum* and *S. pennellii* drought-responsive orthologs. Numbers indicate the count of TFs per family associated with species-specific or stress-ready gene sets.

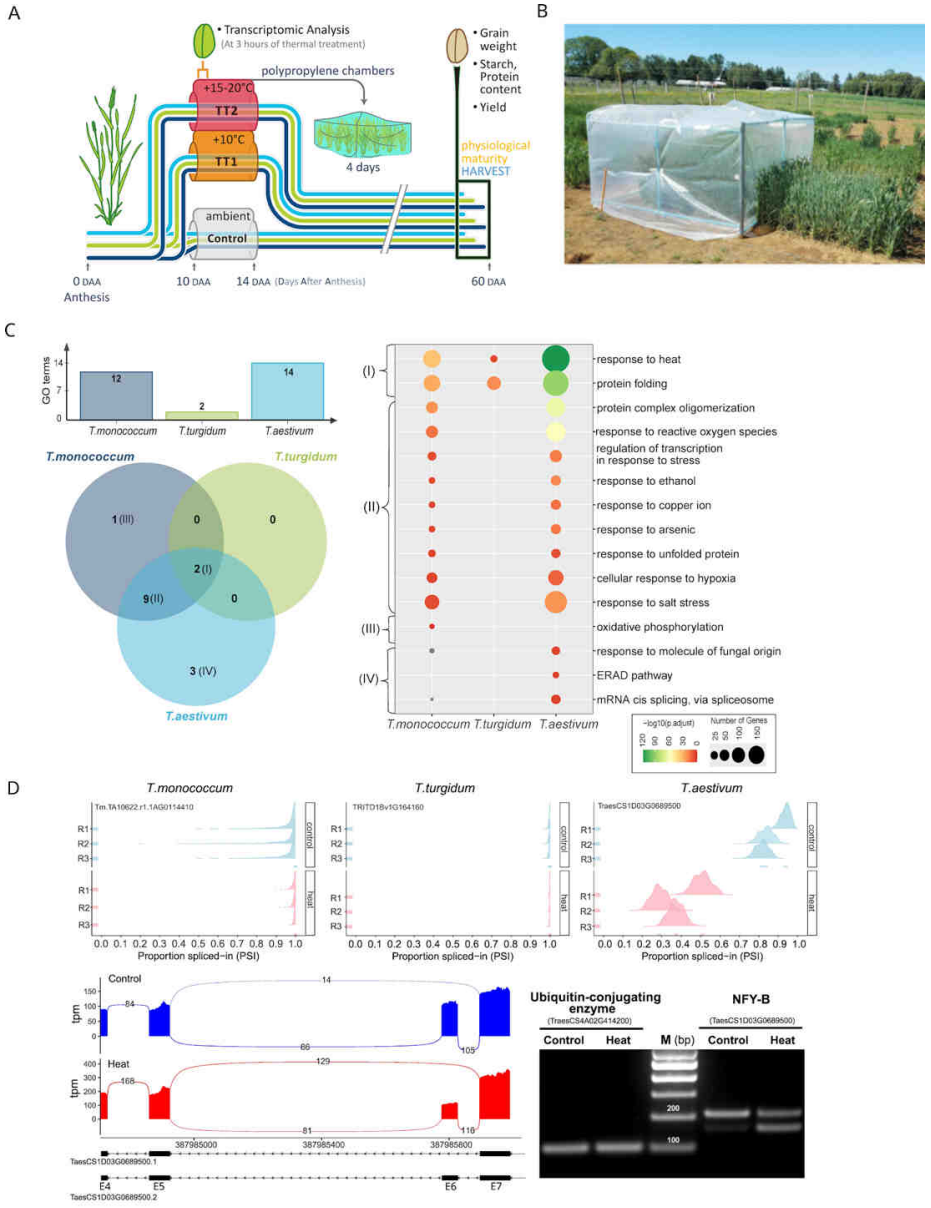


Figure 7. Ploidy-dependent transcriptional and alternative splicing responses to heat stress in wheat.

A) Schematic representation of the experimental design used to assess heat stress responses in wheat species with different ploidy levels (*Triticum monococcum*, diploid; *T. turgidum*, tetraploid; *T. aestivum*, hexaploid). Heat treatments were applied during early grain filling (10-14 days after anthesis, DAA) using polypropylene chambers with two thermal conditions: TT1 (+10°C above ambient) and TT2 (+15-20°C above ambient). Transcriptomic analysis was performed 3 hours after heat exposure (TT2), and physiological traits were evaluated at maturity.

B) Field implementation of polypropylene chambers used for controlled heat stress treatments.

C) Comparative functional analysis of differentially expressed genes in response to heat stress. Left: Venn diagram showing the number of significantly enriched Gene Ontology (GO) biological process terms identified in each species. Right: GO enrichment categories classified into conserved (I) and species-specific (II-IV) responses. *T. aestivum* uniquely exhibited enrichment for mRNA splicing via the spliceosome, suggesting a distinct post-transcriptional regulatory mechanism.

D) Heat stress induces alternative splicing of the NFY-B transcription factor (*TraesCS1D03G0689500*) specifically in *T. aestivum*. Top: Density distribution of percent spliced-in (PSI) values across three biological replicates (R1-R3) for the *NFY-B* gene in *T. monococcum*, *T. turgidum*, and *T. aestivum* under control (blue) and heat stress (pink) conditions. Middle: Sashimi plot visualization of RNA-seq read coverage and splice junction usage for *NFY-B* in *T. aestivum*, highlighting heat-induced exon skipping (exon E6) under heat stress. Read coverage is shown in transcript per million (TPM), with junction read counts indicated. Bottom: RT-PCR validation of *NFY-B* alternative splicing, showing distinct isoform patterns under control and heat stress conditions. A shorter transcript lacking E6 is detected under heat stress. The ubiquitin-conjugating enzyme (*TraesCS4A02G414200*) was used as a non-splicing control.

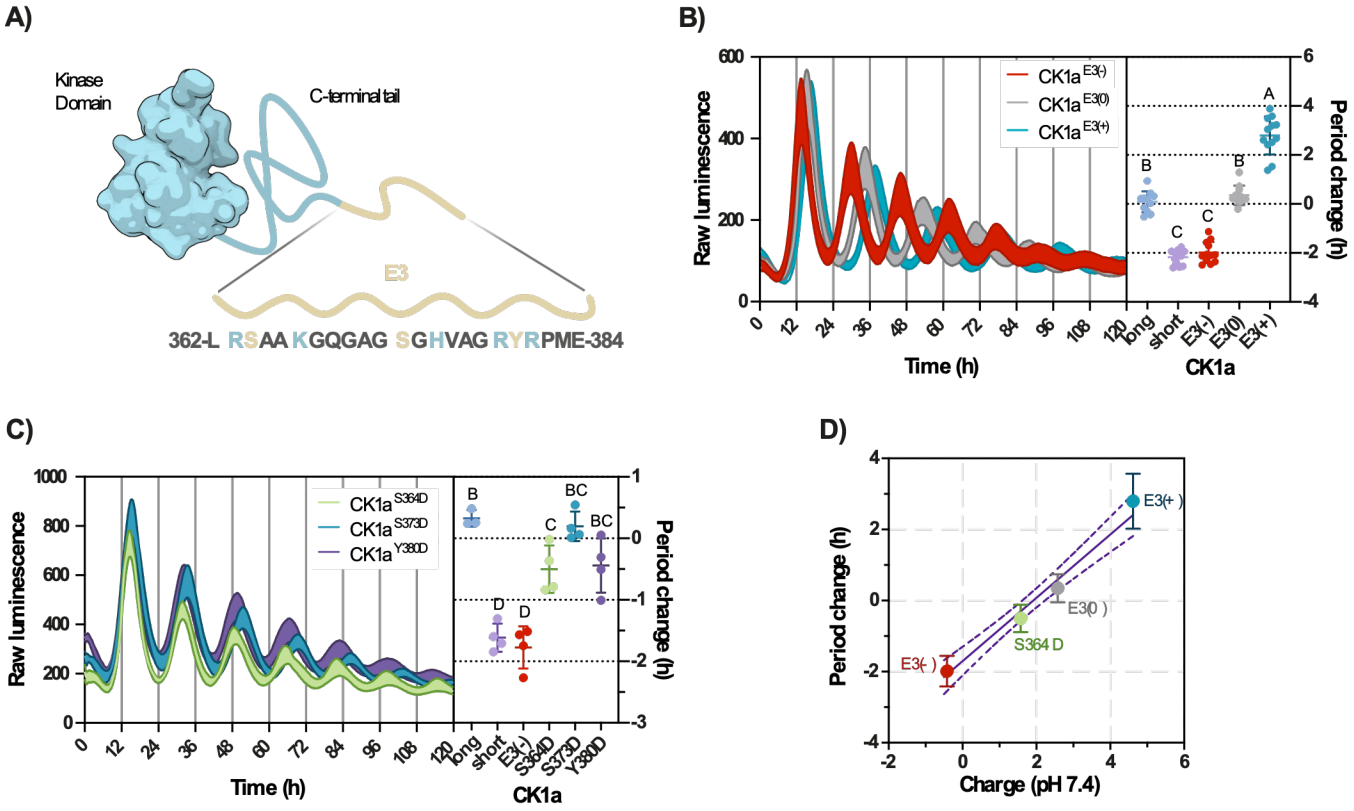


Figure 8. Charges drive the circadian regulatory properties of CK1a E3 region in *N. crassa*. A) Schematic representation of the CK1a protein highlighting the E3 region (last 23 residues) of the C-terminal tail in brown. Within the E3 region, phosphorylatable residues are marked in brown, and positively charged amino acids are indicated in light blue. The sequence analysis reveals that the E3 region has a net positive charge, with phosphorylatable sites flanked by positively charged residues. This structural organization suggests a potential regulatory role for the E3 region through phosphorylation-driven charge modulation. **B)** Representative luminescence rhythms over time and period analysis relative to the *knock-in WT* strain for strains expressing CK1a mutants with altered charges in E3. Mutants were generated by replacing the three phosphorylatable residues (S364, S373, Y380) with residues mimicking negative (aspartic acid), neutral (alanine/phenylalanine), or positive charges (lysine/histidine). The period obtained is directly proportional to the charge, indicating that the regulatory capacity of E3 is influenced by positive charges and can be inhibited through phosphorylation. Statistical analysis was performed using one-way ANOVA. The center line represents the mean, while the error bars represent the standard deviation. **C)** Raw luminescence oscillation over time for single-point phosphomimetic mutants in the E3 region of CK1a and calculated period differences relative to the *knock-in WT* strain. Single charges fail to replicate the full period-reducing effect observed in the triple phosphomimetic mutant. **D)** Period length of full-length CK1a mutants carrying charge-altering substitutions in the E3 region is plotted against the net electrostatic charge of the mutated E3. A linear correlation is observed between net E3 charge and circadian period, supporting a graded electrostatic mechanism of period modulation. The dotted lines indicate the 95% confidence interval of the linear fit calculated from the full set of mutants, overlapping points were removed to improve visualization. Error bars represent the standard deviation from four biological replicates.

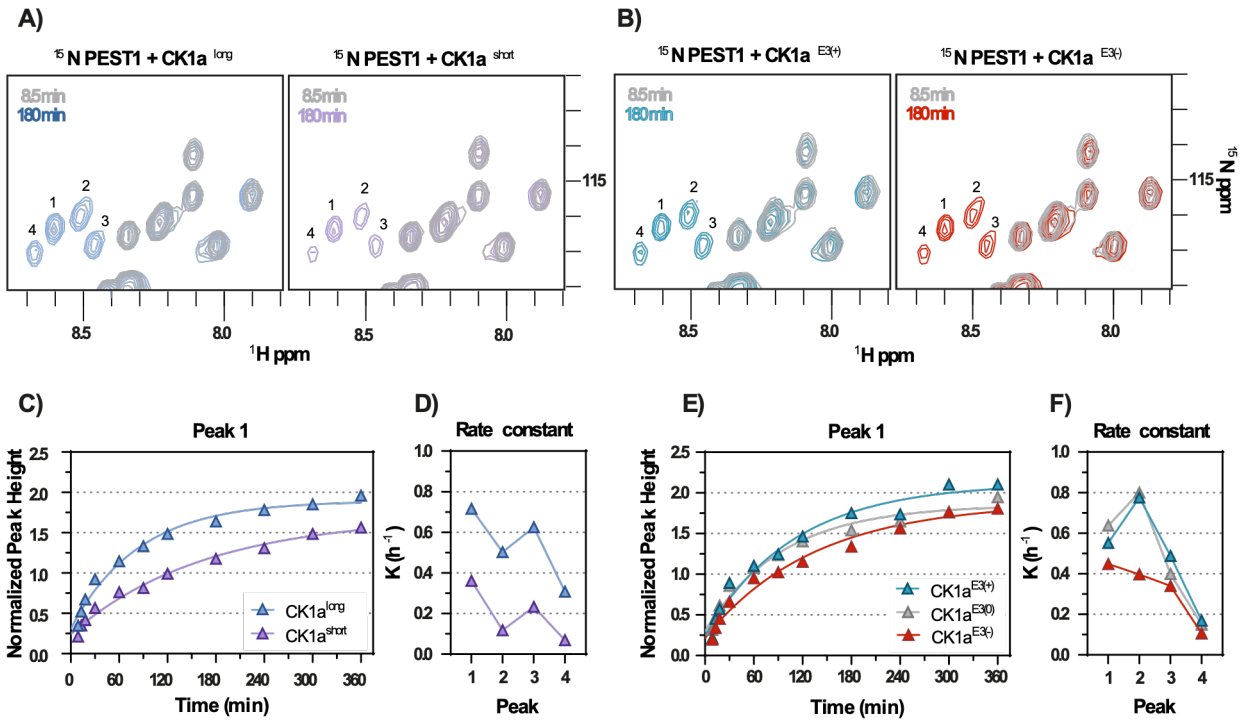


Figure 9. The E3 region modulates CK1a phosphorylation kinetics in a substrate-dependent manner impacting PEST1 processing *in vitro*. (A,B) Representative SoFast NMR assay tracking the phosphorylation of the 15N-labeled PEST1 peptide by CK1a mutants over time. Shown are overlaid spectra of the initial time point (~8.5 min, gray) and the half-time point (~180 min) of the reaction. The assay reveals the sequential appearance of four distinct phospho-peaks, indicating progressive phosphorylation events across a 6-hour reaction. **(C,E)** Calculated peak volumes of the first phospho-peak for the proteins $CK1a^{long}$ and $CK1a^{short}$ and E3 charge mutants with negative, neutral, and positive charges, respectively. **(D,F)** Reaction rates for the four phospho-peaks were calculated from panels A and B, respectively. $CK1a^{short}$ and $CK1a^{E3(-)}$ exhibit slower rates compared to $CK1a^{long}$ and $CK1a^{E3(+)}$ across all phospho-peaks. The results demonstrate how the E3 region modulates the efficiency of CK1a in phosphorylating PEST1 in a charge-dependent manner.

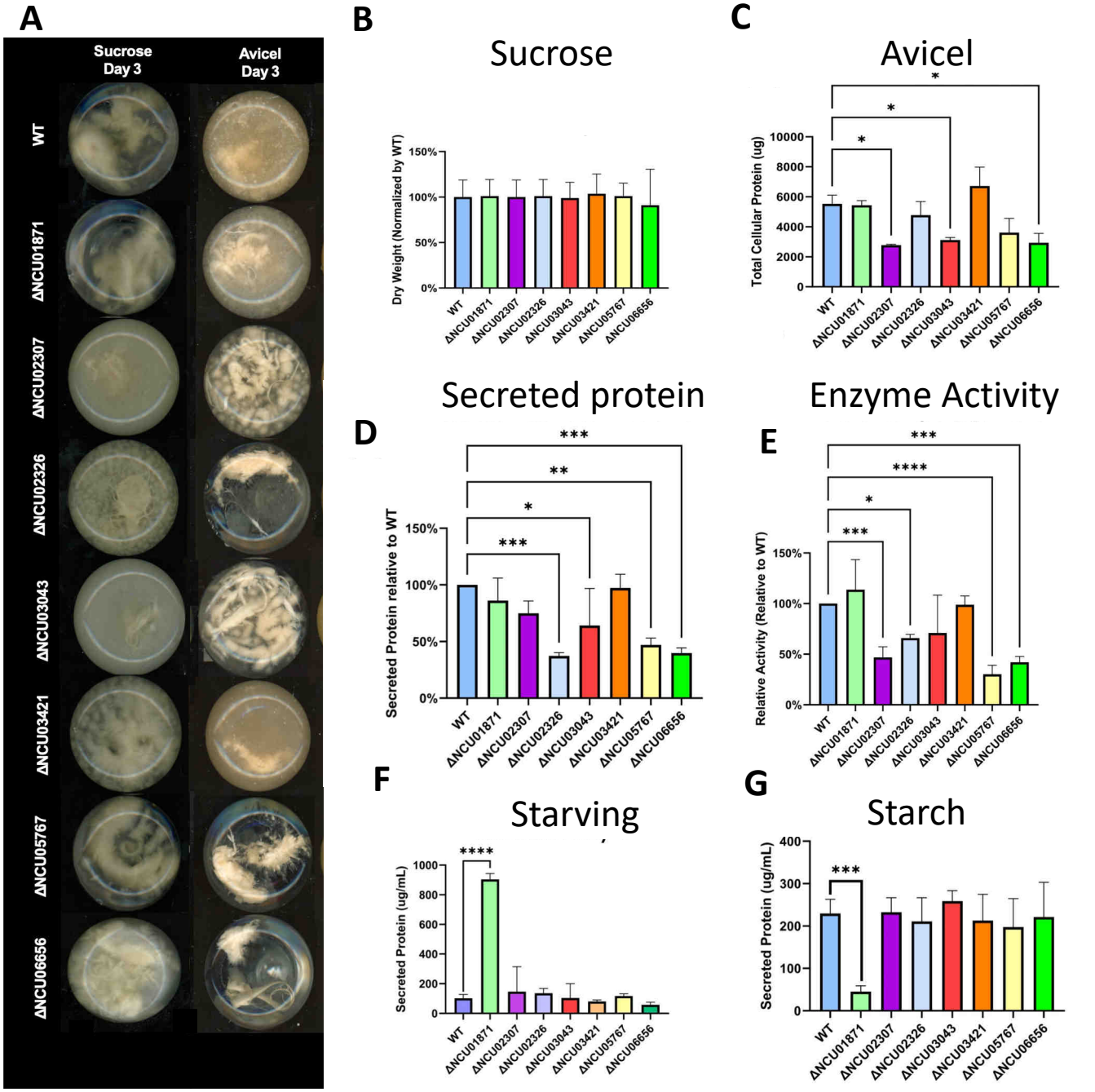


Figure 10. Phenotypic characterization of mutant strains during growth on complex carbon sources.

A) Representative bottom-flask view of wild-type and mutant strains after 3 days of growth in constant light and shaking (200 rpm) using Avicel as the sole carbon source. **B–C)** Quantification of biomass accumulation after 3 days of growth on sucrose (**B**) or Avicel (**C**) as the sole carbon source.

D–E) Medium shift assay: strains were pre-cultured for 48 hours in sucrose medium and then transferred to Avicel-containing medium. Secreted proteins were quantified from culture supernatants (**D**), and enzymatic activity was measured (**E**) after the shift. **F–G)** Quantification of total secreted proteins during growth on minimal medium supplemented with sucrose (**F**) or starch (**G**) as carbon source.

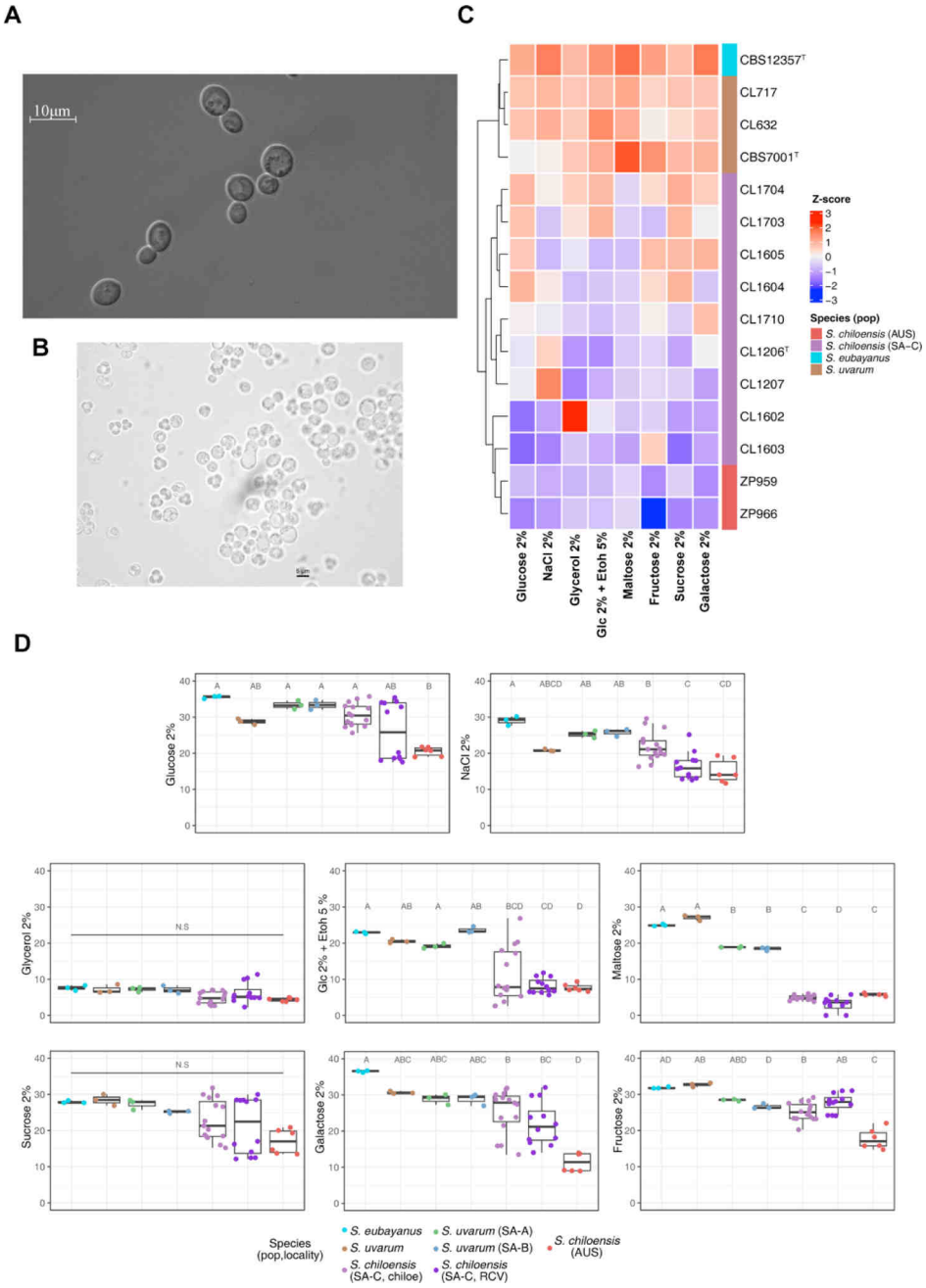


Figure 11. Phenotypic diversity in *S. chiloensis* sp. nov. **A**) Differential interference contrast micrograph of budding cells of *S. chiloensis* sp. nov. grown in YPD broth after three days at 25°C. Bars: 10 µm. This image was obtained using differential interference contrast (DIC) microscopy. **B**) Budding cells and asci with ascospores on Yeast extract—Malt extract agar (YM) after three days at 25°C using 40X microscopy. **C**) Heatmap depicting the phenotypic diversity in *S. chiloensis* sp. nov. obtained from 8 different environmental conditions. Strains are grouped by hierarchical clustering. The colours indicate the species and *S. chiloensis* sp. nov. (SA-C in purple, and AUS in red), *S. uvarum* (brown) and *S. eubayanus* (calypso). The heatmaps were obtained from the Area Under the Curve (AUC) data and normalized using Z-score per column. **D**) The Area Under the Curve (AUC) across all environmental conditions grouped by species, lineages and locality. Different letters reflect statistical differences between strains with a *P*-value < 0.05, one-way analysis of variance (ANOVA). Glc = glucose, EtOh = Ethanol (Extracted from Pena et al., 2024).

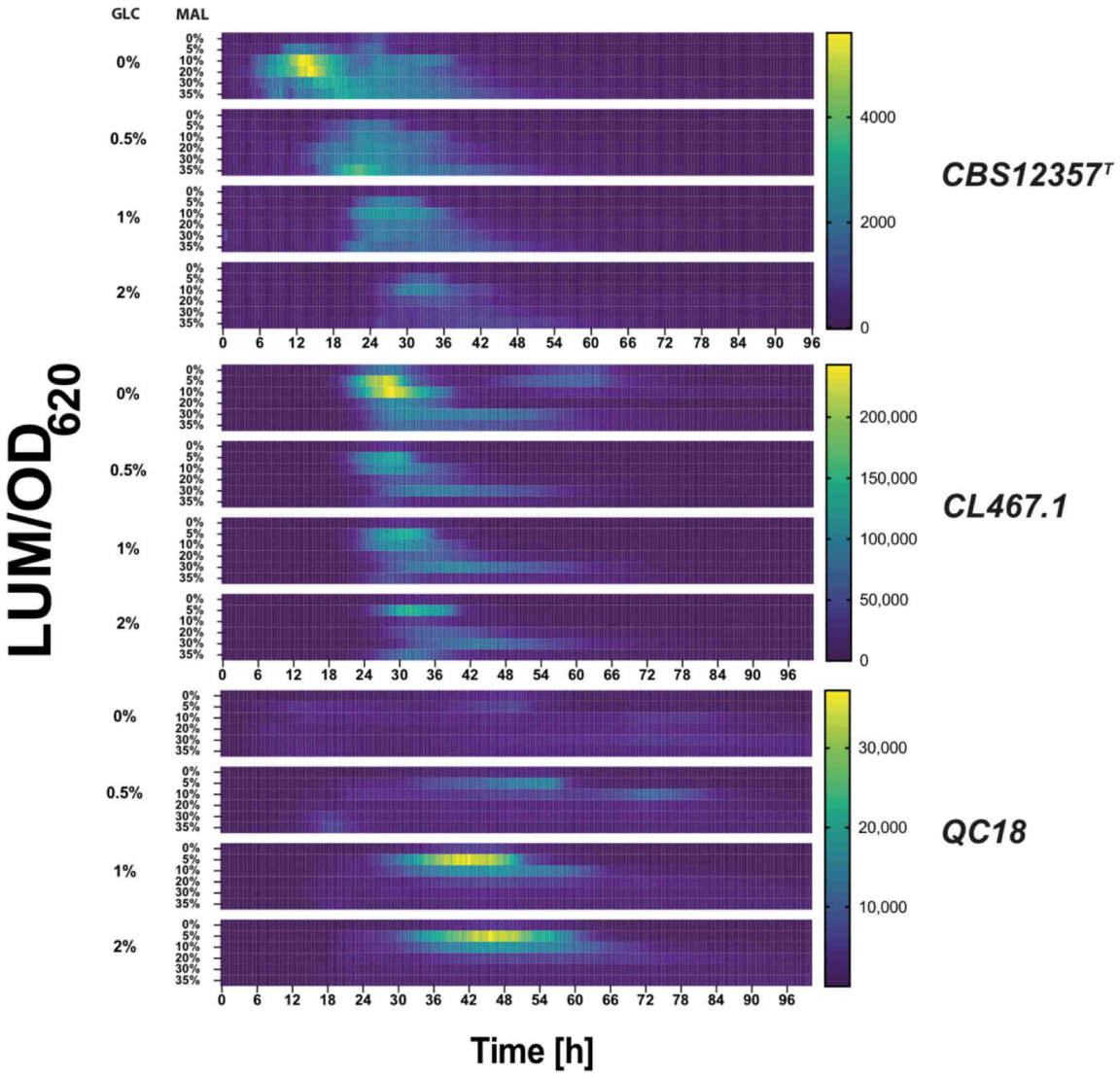


Figure 12. Metabolic transition reporter test under microcultivation conditions. Using a matrix of different glucose (0%, 0.5%, 1%, 2%) and maltose (0%, 2%, 5%, 10%, 20%, 30%) concentrations in YP medium, we evaluated the pMAL32 transcriptional activation in the CBS12357T (upper), CL467.1 (middle), and QC18 (below) *Saccharomyces eubayanus* strains. The luciferase activity is shown in a heatmap as the average luminescence normalized by optic density at 620 nm (LUM/OD620) in arbitrary units for each transformed strain in the glucose-maltose matrix for 100 h. The highest luminescence (or promoter activation) is shown in bright yellow, and inactivation is shown in dark blue in the heatmap.

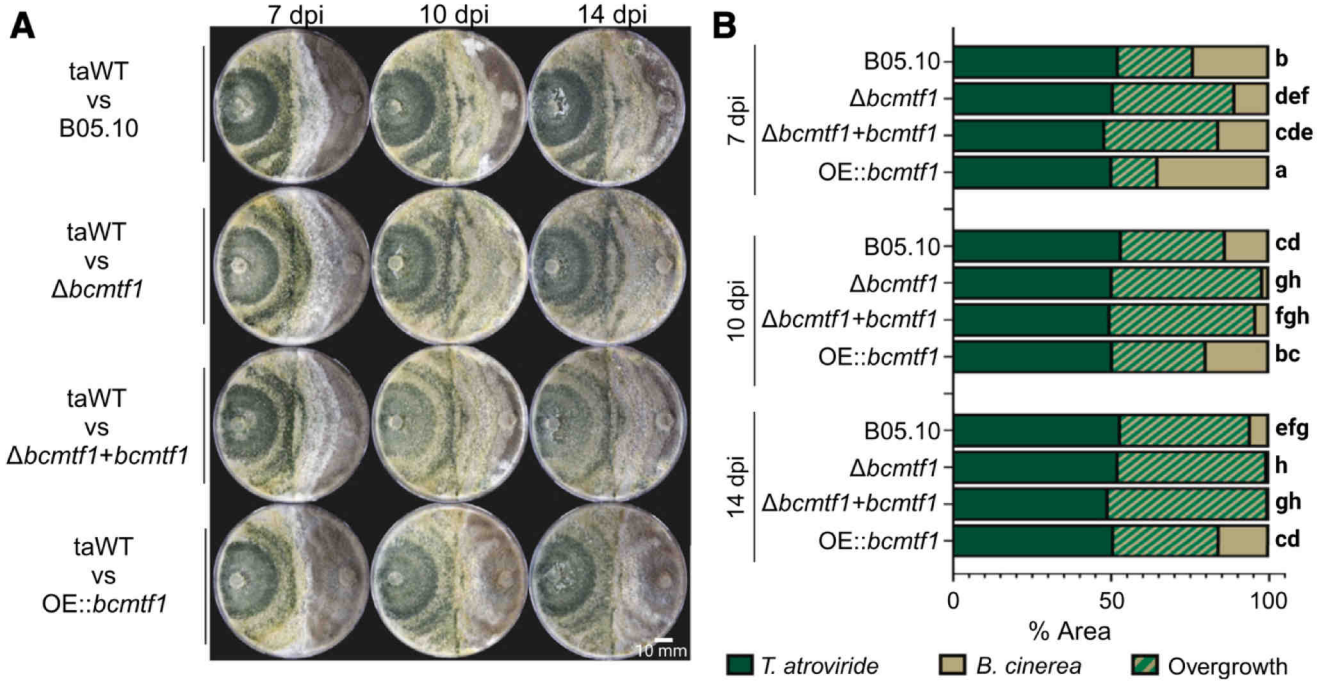


Figure 13. Overexpression of the BcMTF1 TF enhances *B. cinerea's* ability to counteract *T. atroviride* mycoparasitism. A) Confrontation assays were conducted in Petri dishes containing PDA as indicated in the Methods. A control *wild-type/wild-type* interaction (taWT vs. B05.10) is depicted at the top of the figure. A representative picture was selected for *B. cinerea* loss (Δ*bcmtf1*) and gain-of-function mutants (complemented: Δ*bcmtf1*+*bcmtf1,* and overexpression: OE::*bcmtf1*). Each column shows the days elapsed post-inoculation (7, 10, and 14 dpi), whereas rows specify the confronted strains. Both fungi were inoculated as agar plugs, with *T. atroviride* placed on the left side of the plate and *B. cinerea* on the right. Scale bar (bottom right corner) represents 10 mm. **B)** Quantification of the growth area for the phytopathogenic fungus (B05.10, Δ*bcmtf1*, Δ*bcmtf1*+*bcmtf1*, and OE::*bcmtf1*) and the biocontroller, and the observed overgrowth of the latter as depicted in (a). Distinct letters denote statistically significant differences ($p < 0.05$).

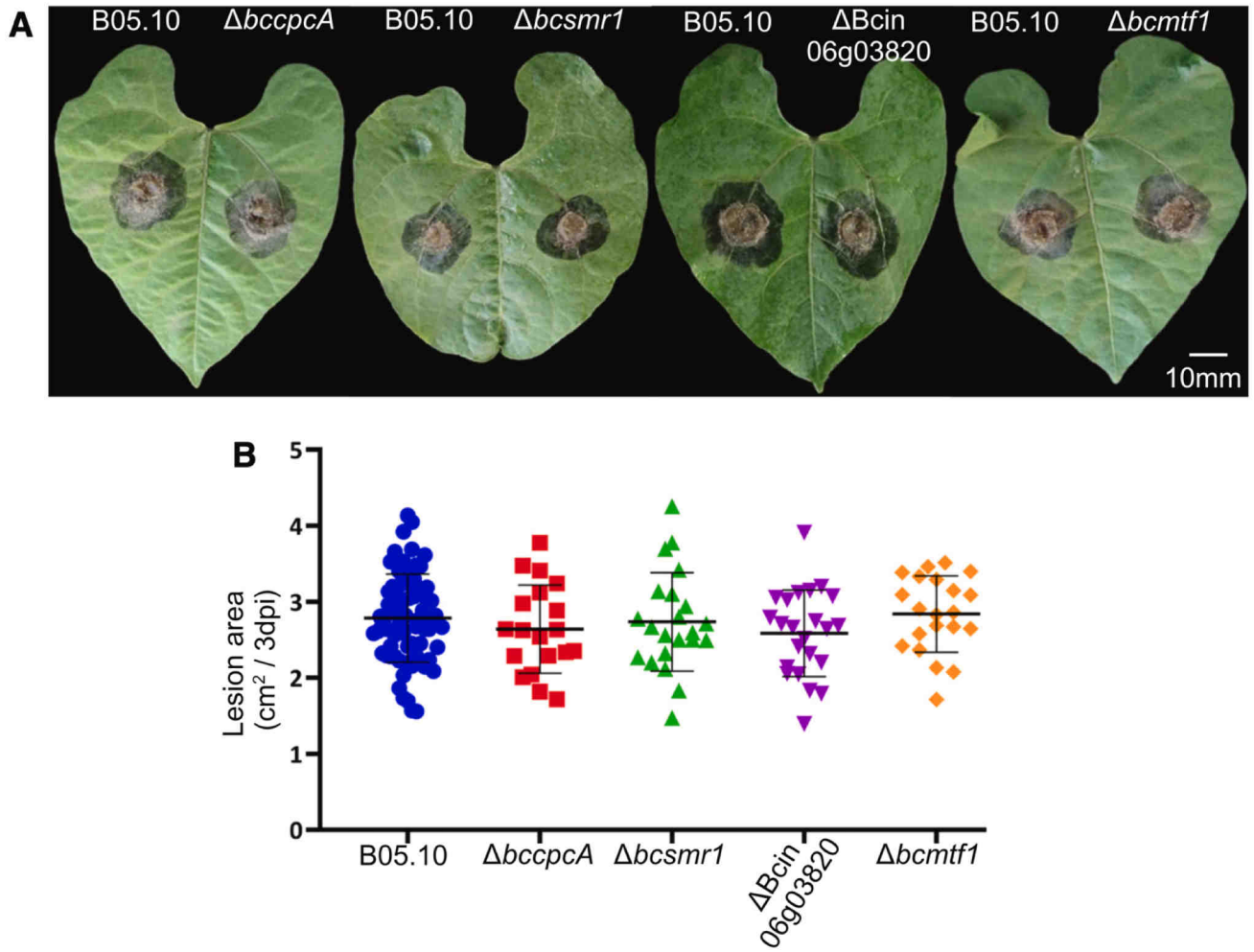


Figure 14. Loss-of-function mutants of each *B. cinerea* TF show no significant lesion variation in their ability to cause infection on *P. vulgaris* leaves. A 6 mm agar plug of each *B. cinerea* strain was inoculated on one-week-old *P. vulgaris* leaves and kept inside plastic boxes at 20 C for 72 h (3 dpi). **A**) Representative images of the virulence assay conducted on bean leaves. *B. cinerea* B05.10 was inoculated on the left section of each leaf, whereas TF mutants were placed on the right, as indicated in each case. Scale bar represents 10 mm. **B**) The necrotic lesion area (mean values ±standard error; black lines) was assessed after 3 dpi. No significant differences were observed.

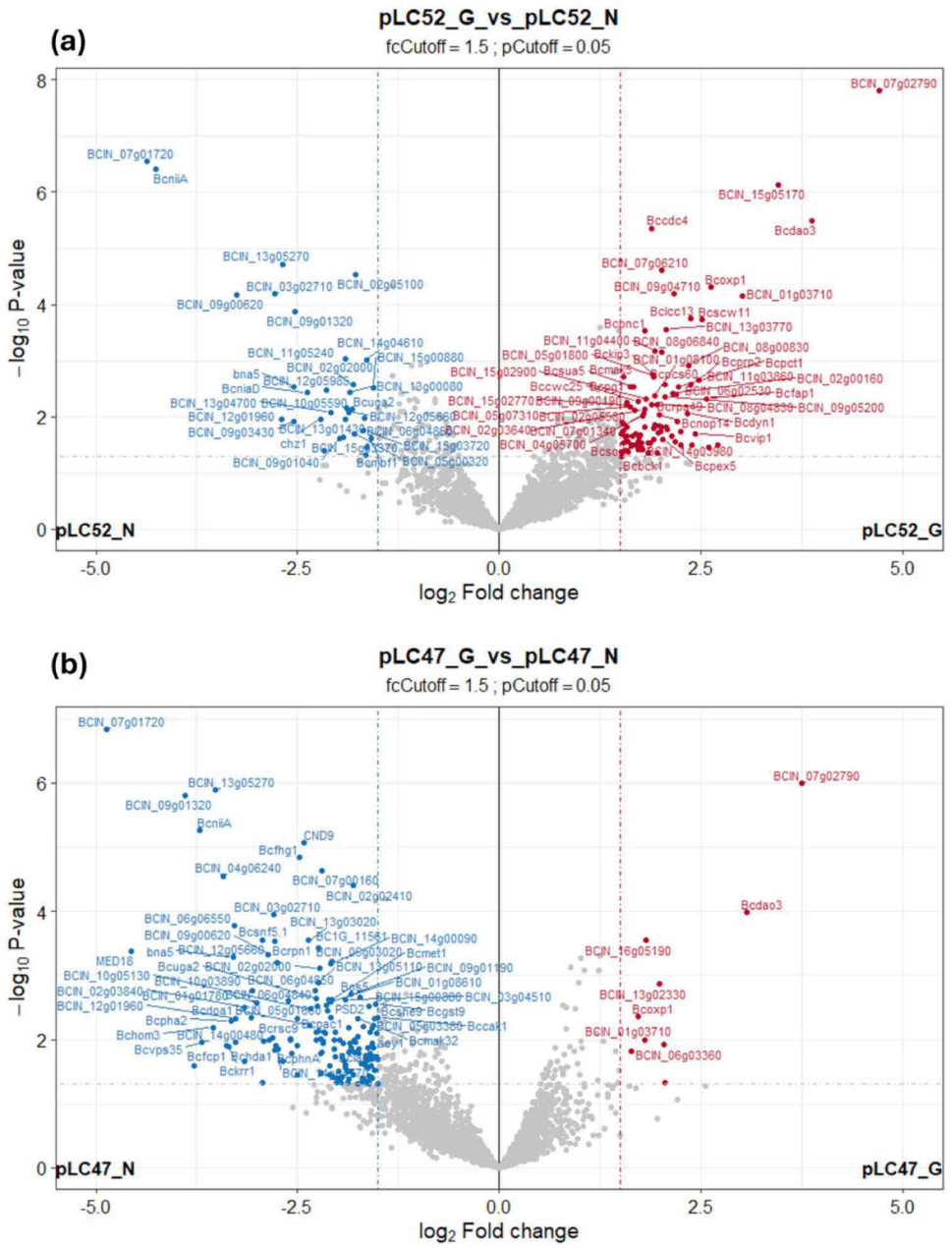


Figure 15. Volcano plots showing significantly enriched (Log2FC >1.5, p = 0.05) proteins under different nitrogen conditions for **(A)** the bcFRQ1-TurboID expressor line (pLC47) and **(B)** the TurboID control strain (pLC52). The significant proteins shown are not adjusted for multiple comparison.

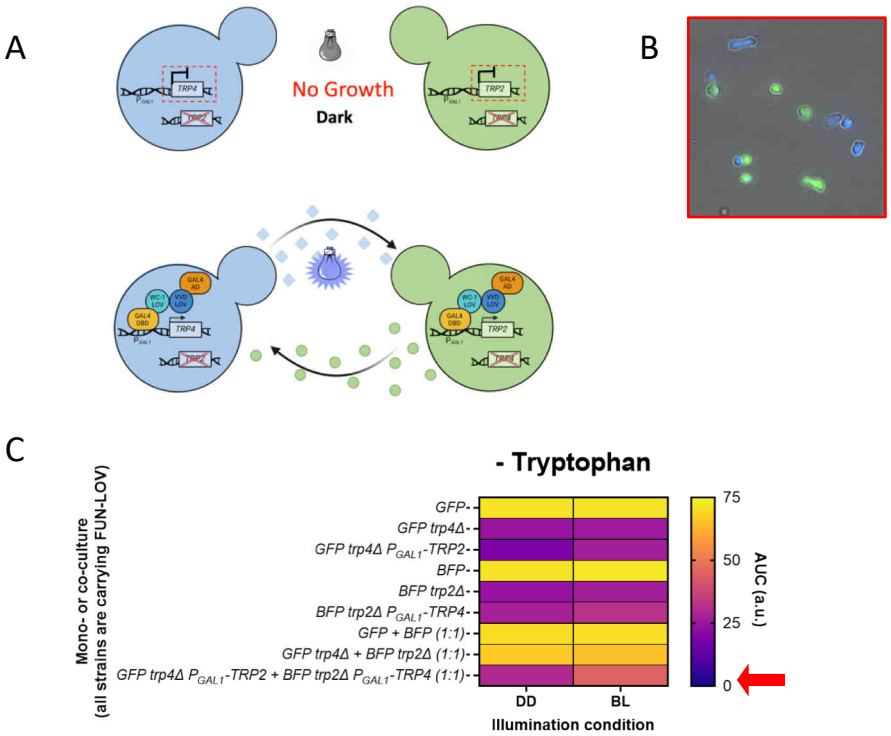


Figure 16. Optogenetic control of syntrophic growth in engineered yeast co-cultures. **A**) Yeast strains with complementary auxotrophies for *TRP2* (green) and *TRP4* (blue) are engineered to express the missing tryptophan biosynthesis genes under control of the light-activated FUN-LOV system. In darkness (top), neither strain expresses the required gene, preventing cross-feeding and resulting in no growth. Under blue light (bottom), FUN-LOV is activated in both strains, inducing *TRP2* and *TRP4* expression, which enables production and reciprocal exchange of pathway intermediates (green and blue dots). This optogenetically induced metabolic complementation allows syntrophic interaction and cooperative growth dependent on illumination.

B)The strains also carry fluorescent markers so the two different strains, in terms of relative abundance and spatial distribution can be tracked in the pseudo community.

C) Heat map illustrating the growth of the strain of the indicated genotypes in darkness (DD) and in blue-light (BL). The arrow highlights the syntrophic community supporting growth in light but not in the dark.

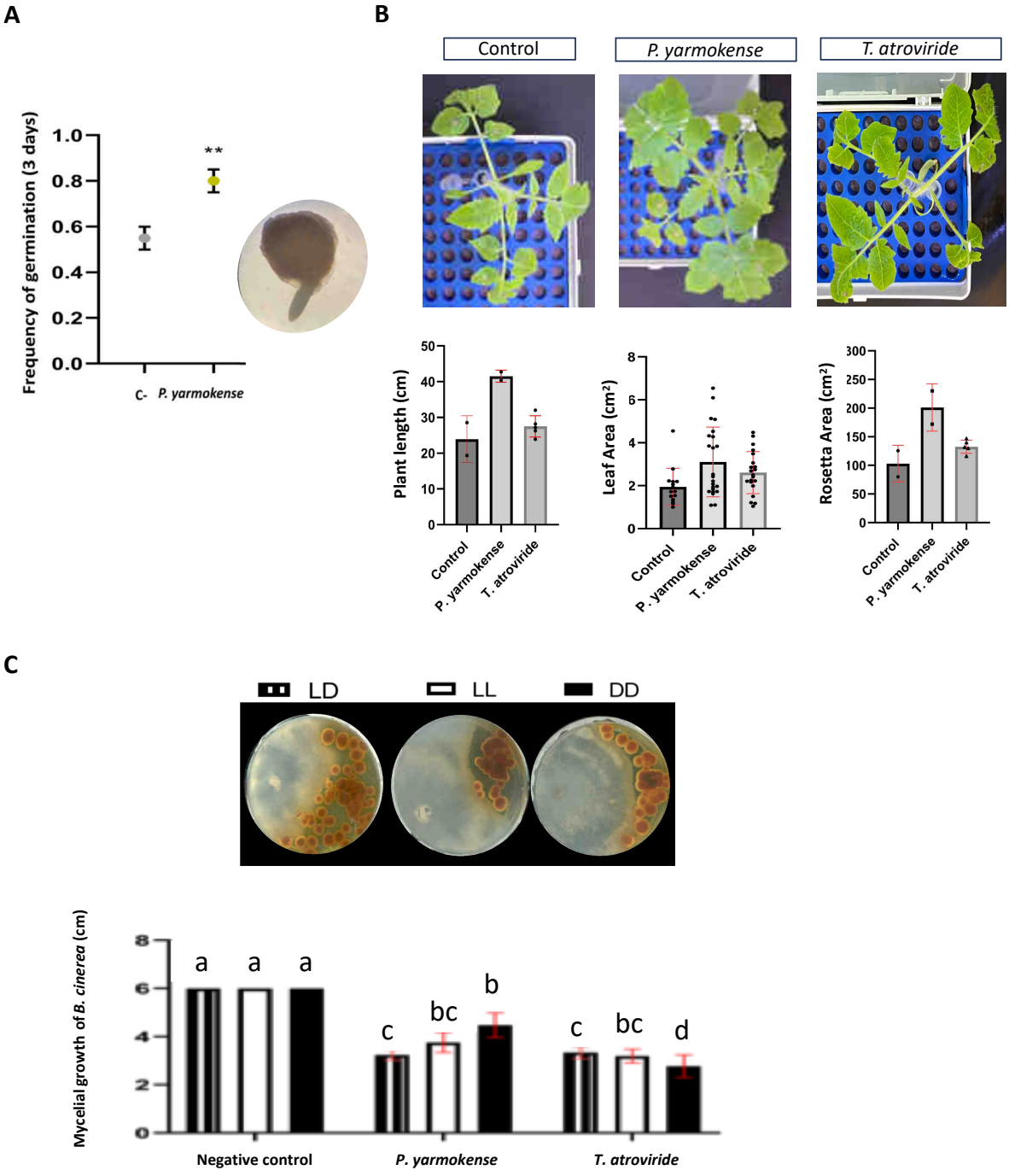


Figure 17. Effect of *P. Yarmokense on S. lycopersicum and B. cinerea.* *P. yarmokense* promotes the germination of *S. lycopersicum* (**A**) and enhances various plant growth parameters (**B**). Additionally, it inhibits the growth of *B. cinerea* in confrontation assays—a trait modulated by environmental cues such as light availability (**C**).

A B

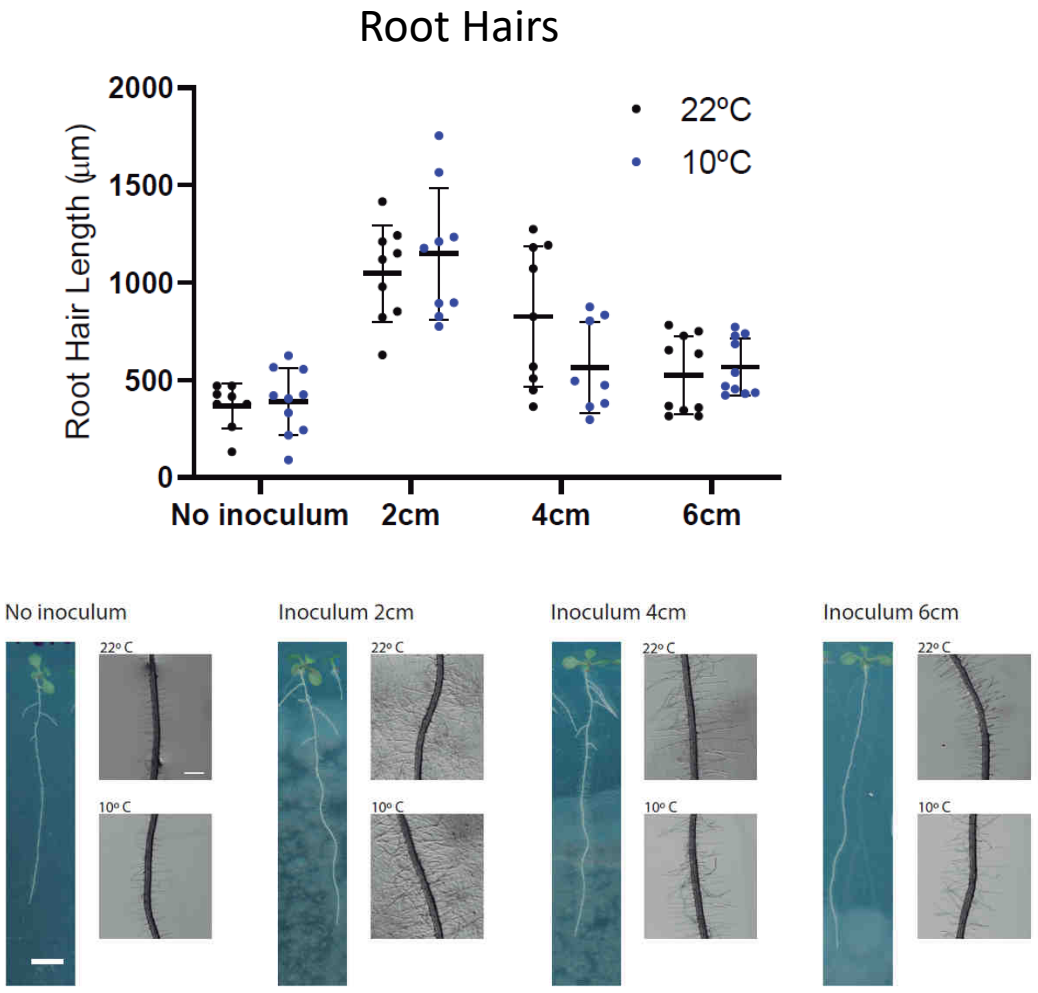


Figure 18. A *Humicolopsis. spp* isolate promotes root hairs development. The fungus was inoculated at different distances from the seedlings, as indicated , and the root hair lengths was quantified. The plant-fungi co-inoculations were monitored at either 10 or 22 C.

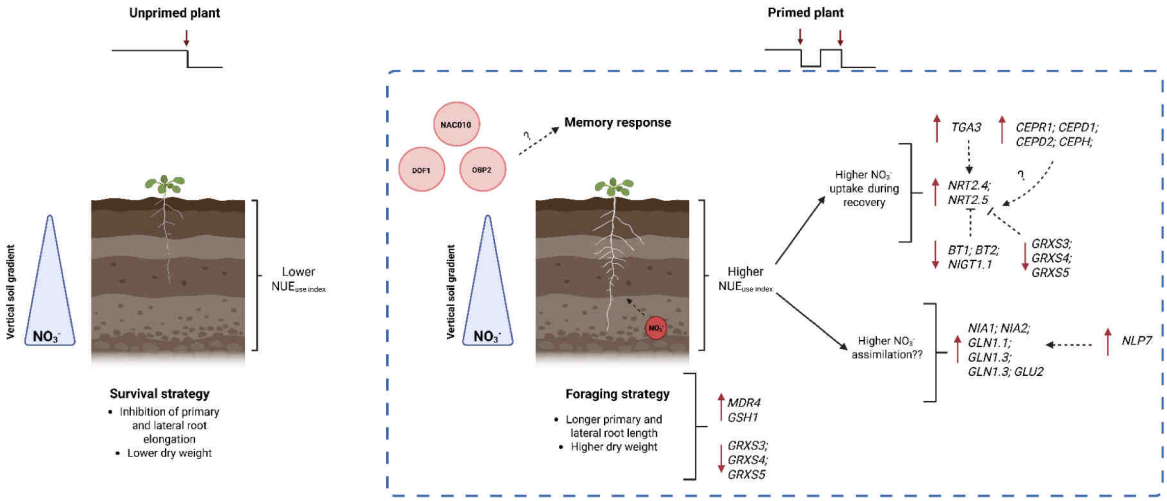


Figure 19. Molecular memory in response to fluctuations in nitrate availability in *A. thaliana.* Priming alters the response to the triggering stimulus. Upon exposure to the triggering stress, primed plants exhibit a different response pattern when compared to unprimed plants. For instance, when subjected to 72h of triggering stimulus, primed plants grow longer primary and lateral roots, whereas unprimed plants experience inhibited primary and lateral root elongation. This improved root system architecture in primed plants likely supports an optimized nitrate interception by the roots, which can be advantageous under N-deficient conditions, allowing the plant to exploit deeper soil layers where nitrate diffusion occurs. Interestingly, primed plants show higher dry weight than unprimed plants, despite potential resource allocation associated with maintaining this memory response. Furthermore, memory influences Nitrogen Use Efficiency (NUE), as primed plants exhibit a higher NUE use index. During memory phase, primed plants also demonstrate increased nitrate uptake capacity, suggesting priming enhances the ability to efficiently absorb nitrate upon re-exposure to the nutrient. RNA-seq analysis reveals that, during memory phase, primed plants show an upregulation (upward-pointing arrows) of genes encoding high-affinity nitrate transporters and some of their positive transcriptional regulators, along with a downregulation (downward-pointing arrows) of negative regulators. Additionally, upregulation of genes involved in nitrate assimilation in primed plants suggests an improved ability to assimilate nitrate. Our systems biology gene regulatory network analysis identifies NAC010, DOF1 and OBP2 transcription factors as key hubs mediating memory response. Current analyses aim to further elucidate their specific roles in mediating this memory mechanism.

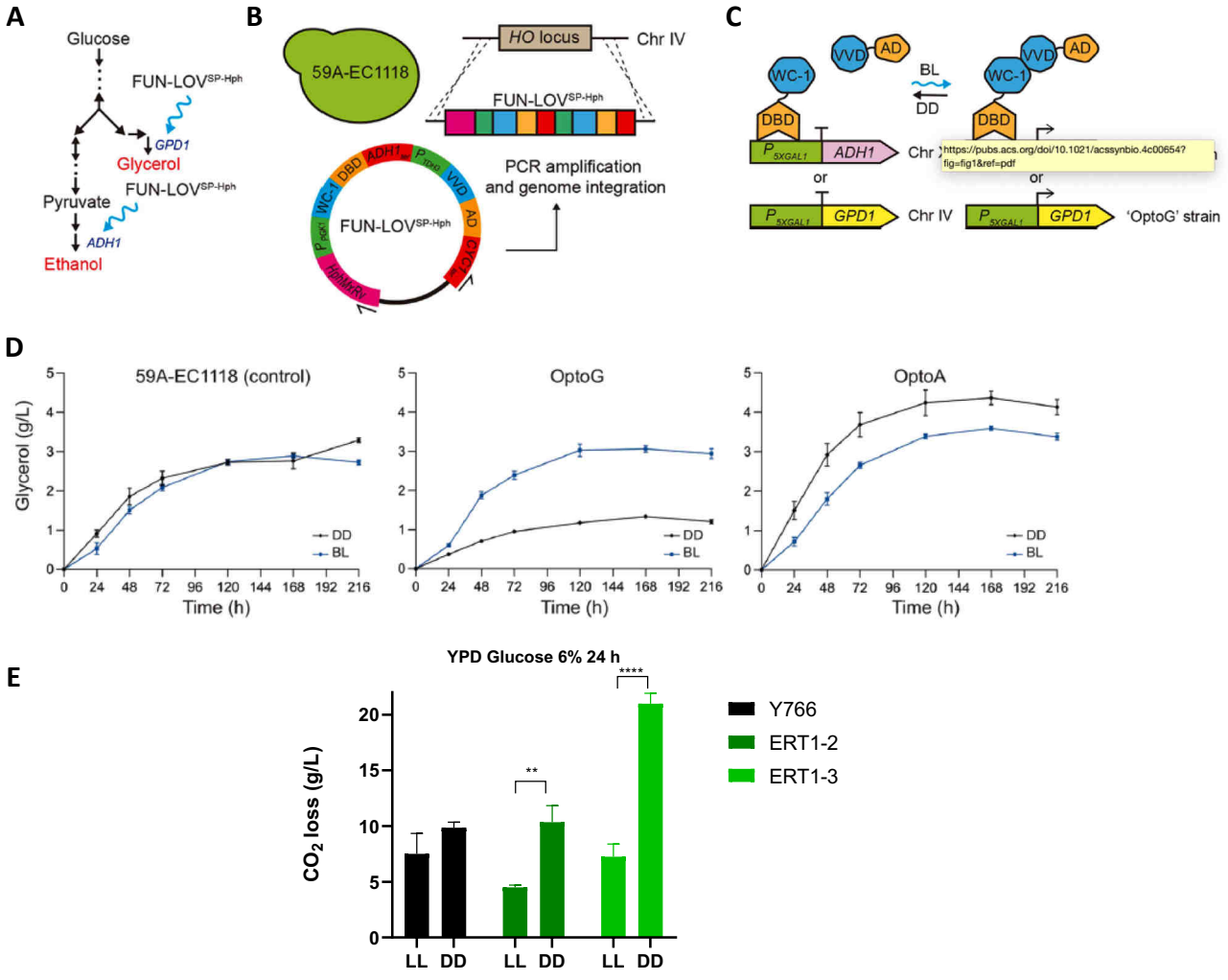


Figure 20. Optogenetic control of the glucose fermentation pathway in yeast. A) FUN-LOVSP-Hph optogenetic system was used to control (by BL) two genes of the glucose fermentation pathway: ADH1 or GPD1. **B**) FUN-LOVSP-Hph is encoded in a SP and was integrated into the HO locus of the 59A-EC1118 wine yeast strain. **C**) Endogenous promoters of ADH1 and GPD1 were independently swapped by the 5XGAL1 promoter (P5XGAL1), generating "OptoA" and "OptoG" strains, respectively. This enabled the upregulation of the target genes by FUN-LOVSP-Hph upon BL stimulation, which is reversible under dark (DD) conditions. Abbreviation: HphMxRv, hygromycin cassette in the reverse direction; PPGK1, promoter of the PGK1 gene; DBD, DNA-Binding Domain from the Gal4 protein; WC-1, LOV domain from the White Collar-1 protein; ADH1ter, transcriptional terminator of the ADH1 gene; PTDH3, promoter of the TDH3 gene; VVD, LOV domain from the Vivid protein; AD, activation domain from the Gal4 protein; CYC1ter, transcriptional terminator of the CYC1 gene. **D**) Light-controlled glycerol production in the engineered strains. **E**) Additionally, in a different strain, we have used FUN-LOV to control the expression of the TF ERT1, confirming that fermentation can be suppressed by light (when ERT1 is expressed), and allowed in the dark ERTT1).

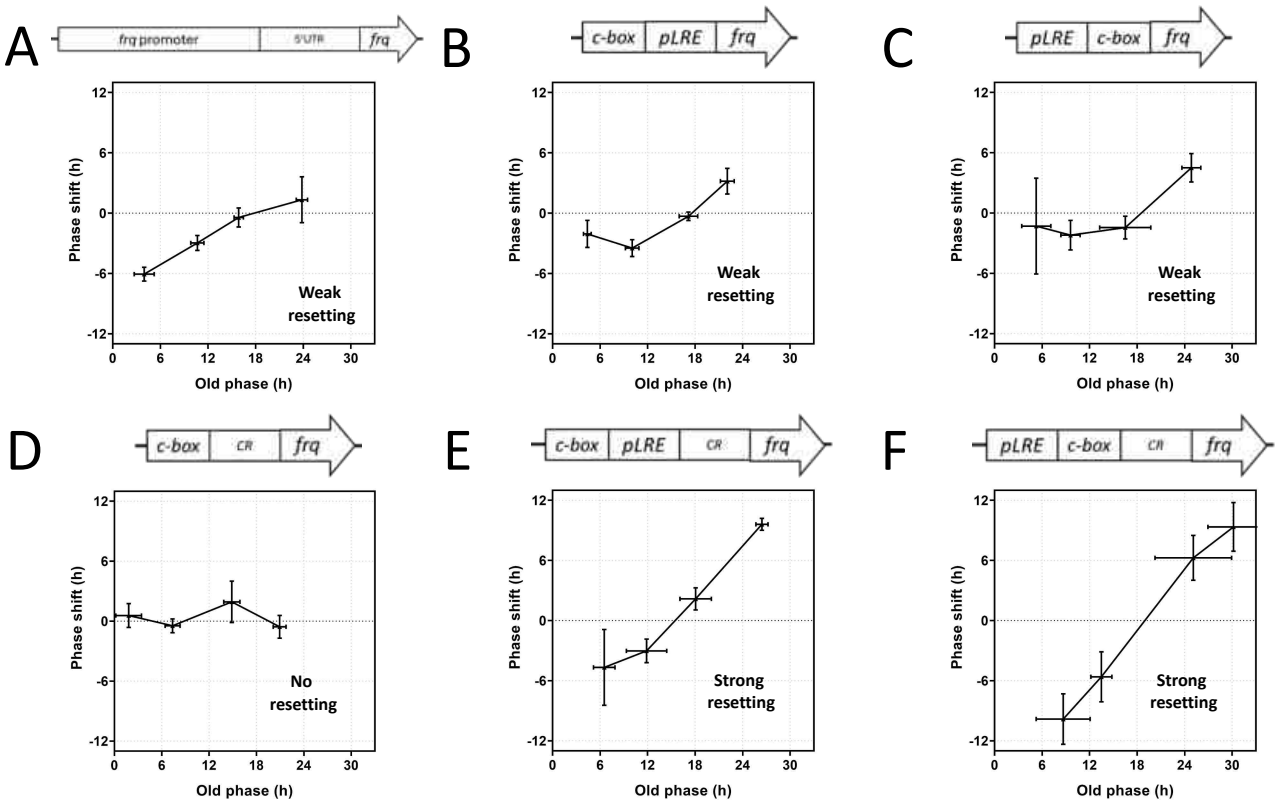


Figure 21. Semi-synthetic oscillators based on composite promoters exhibit differential sensitivity to light pulses.

(A–F) Phase response curves (PRCs) showing the phase shift observed in (A) the WT strain and (B–F) the different oscillators after a 30-minute light pulse (LP), as a function of the circadian time at which the stimulus was applied (old phase). PRCs with weak resetting exhibit phase shifts oscillating around 0 (gray dotted line, Type-1 resetting), whereas strong resetting is characterized by a steep slope, Type-0 resetting (Roenneberg et al., 2003). Strains were synchronized for three days using 22:28 °C thermocycles and 12:12-hour light-dark cycles in four separate incubators to establish four distinct phases before being transferred to DD, at which point luciferase monitoring began. After 48 h in DD, a 30-minute LP was administered, such that the strains were at different circadian times (CTs) at the moment of the pulse. The frqc-box+pLRE:lucPEST construct was used as the reporter. Each value represents the mean ± SD of two to four biological clones with three independent wells each, and reflects the behavior observed in two independent experiments.

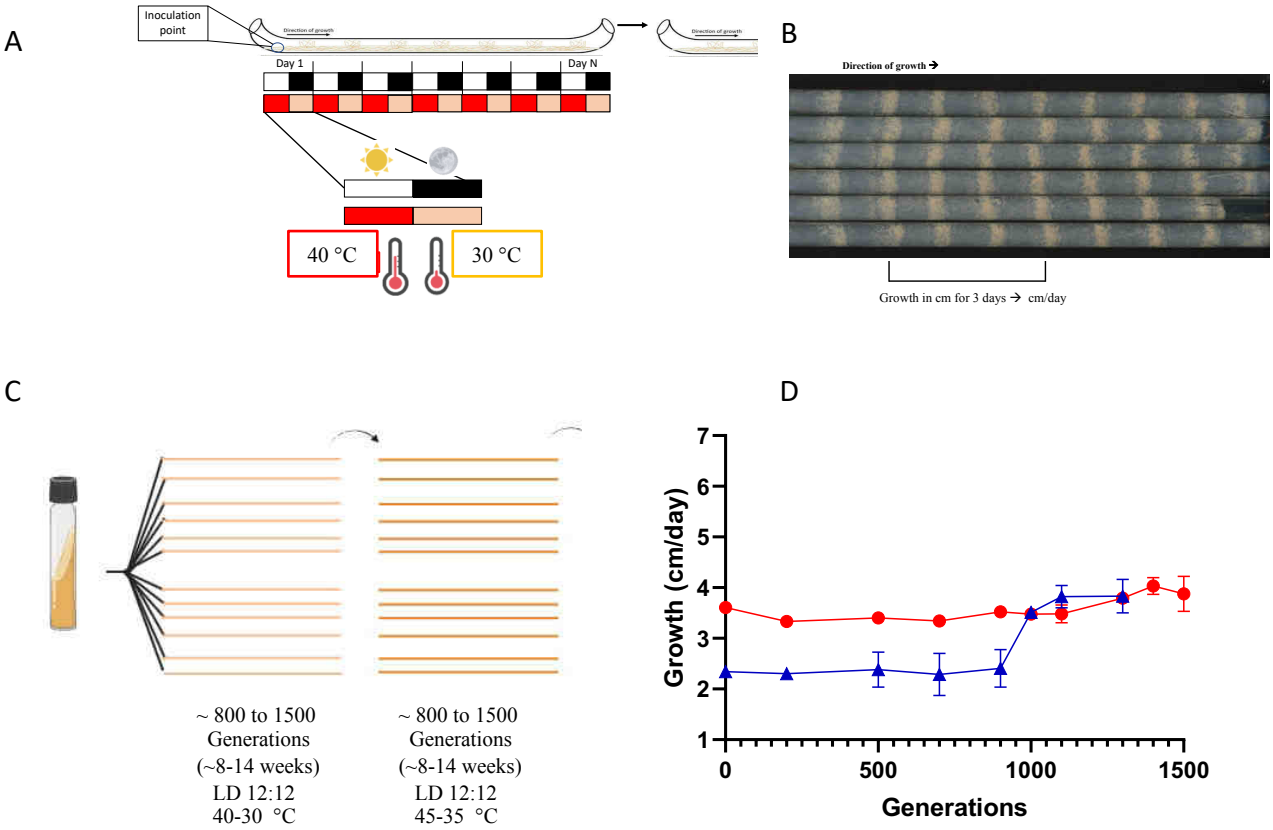


Figure 22. Experimental Evolution under a 12:12 hours 30:40 °C regime. A) Scheme of the Experimental Strategy, where strains are inoculated in race tubes (hollow glass tubes filled with media), which are then kept under 12:12 hours light/dark cycles, which are in synchrony with high/low temperature cycles (i.e 35: 30 °C), mimicking hot days cooler nights. After several days the inoculated strain reaches the end of the race tube, time at which a witness sample is stored at -20 C, and another one is used to inoculate a new race tube. **B)** Photograph of a race tube pack (6x) containing a WT strain growing under the 30:40 ° C regime, where conidial bands tend to occur each day at the end of the cool phase. **C**) After completing ~1500 generations, (~100/ race tube), the strains are now grown under more stringent conditions, where days become hotter, and the protocol is repeated. In each case 12 pseudo replicas (12 race tubes) are grown for each genotype (WT or Rewired). The next graph focuses on strains that were directly evolved in a 30:40 °C DL regime.
B) Naïve strains (meaning that they have not been subjected to experimental evolution) were kept for 1500 generations under the 30:40 °C DL regime. As observed therein, the rewired strain exhibited from the beginning higher growth rate, whereas the WT increased it growth around generation 800. Red circle= WT, blue triangle= Rewired strain (*hsf-1* under the control of a clock and light-regulated promoter. Growth rate was calculated by measuring the linear growth on race tubes.

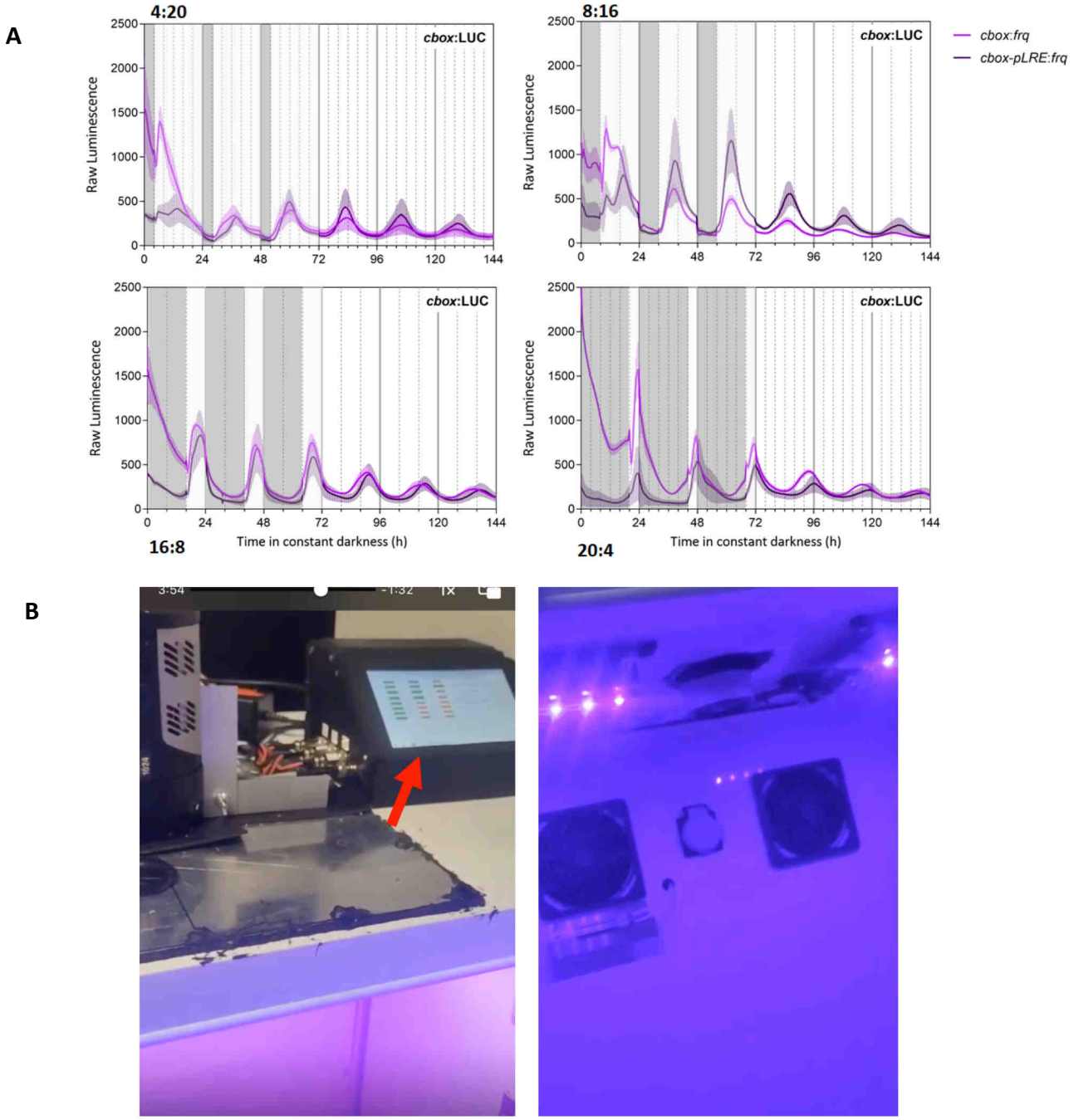


Figure 23. Real-time analysis of clock function under light–dark cycles.
A) The graphs show raw luminescence from reporter strains in which luciferase is driven either by a core clock cis-element (*c-box*) or by a composite promoter responsive to both clock and light inputs (*c-box+pLRE*). As shown, the shape and amplitude of the luminescence traces vary depending on the imposed light regime (gray: darkness; white: light). Notably, the *c-box+pLRE-luc* reporter exhibits strong induction upon light onset. **B**) These recordings were enabled by the use of the LightCycler, a custom-built device (see red arrow) that permits programmable light–dark cycles while capturing bioluminescence data using a CCD camera. Importantly, not inly the light regime can be modified, but also the spectra composition (the image on the right shows blue-light being emitted), and also the intensity of the light.

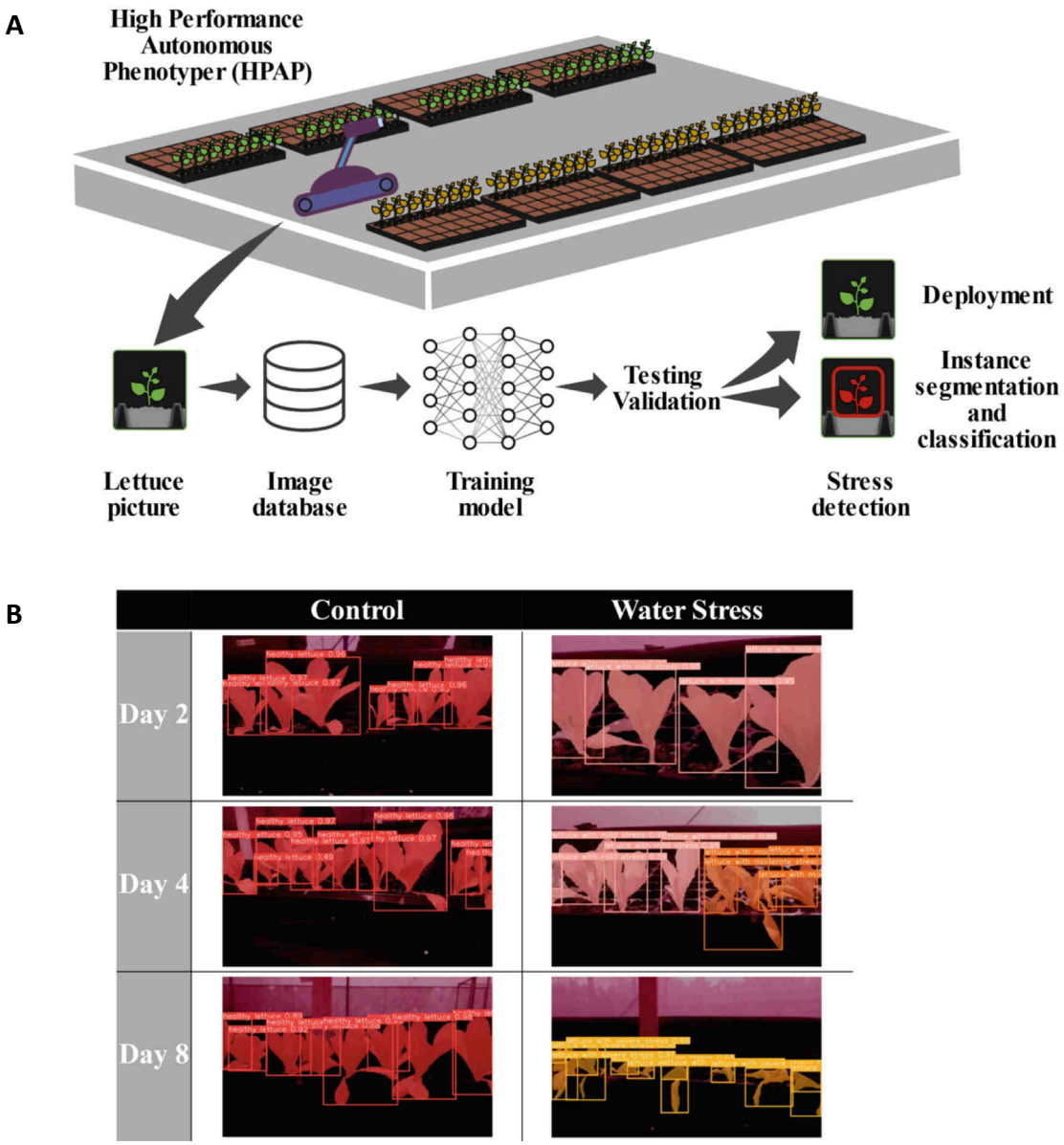


Figure 24. Experimental design for WS-YOLO development. A) The experimental setup involved growing lettuce seedlings under controlled greenhouse conditions, with two groups: a well-watered control and a drought-stressed experimental group subjected to an 8-day water deficit. An autonomous Raspberry Pi-based robot was developed to collect IR images using a Sony IMX219 NoIR 8-megapixel camera. The robot was equipped with ultrasonic sensors for precise navigation, ensuring consistent imaging conditions. This process generated a dataset of 2,119 IR images, further augmented through transformations such as flipping, rotation, zooming, and pixel noise addition to improve model robustness. For stress detection, the dataset was annotated into four categories: healthy, mild stress, moderate stress, and severe stress, based on morphological traits like leaf wilting and reduced turgor. The YOLOv8 deep learning architecture was employed with an instance segmentation approach to train the WS-YOLO model. The training was conducted using the PyTorch framework, optimized with the AdamW optimizer, with a learning rate of 1×10^{-5} over 25 epochs. Hyperparameters were fine-tuned to balance model accuracy with computational efficiency
B) WS-YOLO detection on control and water stress groups throughout the duration of the experiment.

Figure 25. Local Efforts, Global coverage. Interview in Science about the local production of reagents as an alternative to the centralized production model, which is prone to supply disruptions. The iBio scientist Severine Cazaux in the pic during our PME activity).

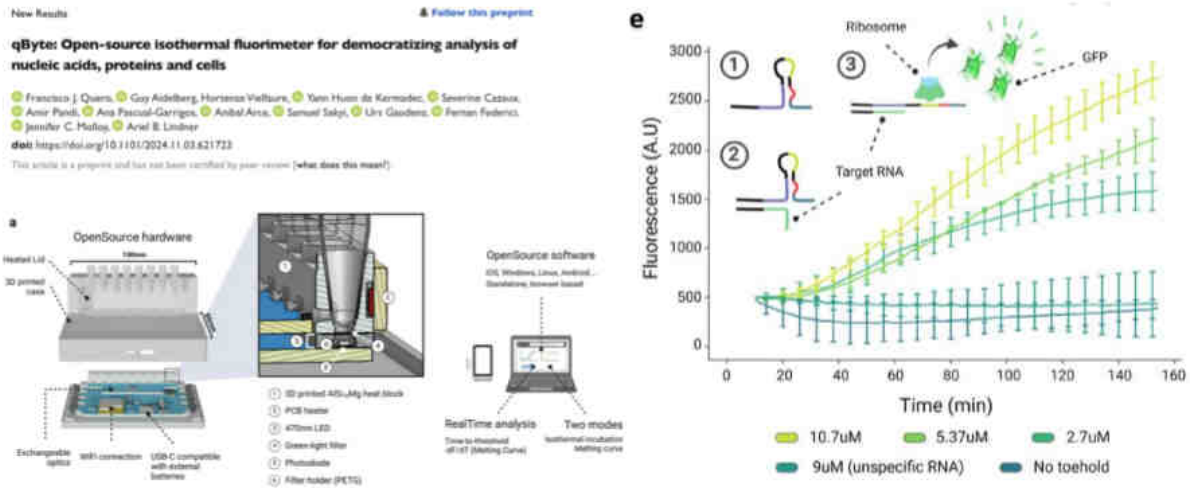


Figure 26. qByte a new open source device created for LAMP reactions in remote settings. This device is being used for cell free reactions and environmental monitoring in remote areas by the FF group (right).

Figure 27. Capacity building with park rangers the use of open hardware microscope in Karukinka Park. Photographs taken in the Chilean Patagonia, as part on one of our PME activities.