Photo: MPIX-TURE (AdobeStock)

11

Genomics applied to climate change

Biotechnology for digital agriculture

Juliana Erika de Carvalho Teixeira Yassitepe | Ricardo Augusto Dante | Isabel Rodrigues Gerhardt | Fernanda Rausch Fernandes | Rafael Soares Correa de Souza | Jaderson Silveira Leite Armanhi | Viviane Cristina Heinzen da Silva | Ana Paula Ribeiro | Márcio José da Silva | Paulo Arruda

Introduction

The most conspicuous manifestations of climate change are higher global atmospheric temperatures, which are already observed in several regions of the world. The projected climate scenarios for the coming decades show increases in the frequency and intensity of extreme events, such as prolonged periods of heat and drought, heavy rainfall, floods, and others (Mbow et al., 2019). Food production is particularly at risk in tropical, subtropical, and semi-arid regions, such as those in South America, Asia, and Africa. Between 1981 and 2010, reductions in the world average productivity were attributed to climate change, especially in these regions. Corn, wheat, and soybeans were reported to lose 4.1%, 1.8%, and 4.5%, respectively (Iizumi; Ramankutty, 2016). Impacts on fruit, vegetable, and animal production are also predicted for these same environments. Climate change not only jeopardizes world food security but also reduces food production and availability. Climate affects several biological processes important to the growth and development of plants and animals, and changes in these mechanisms can alter growth and reproduction rates, as well as nutrient quality and content (Damatta et al., 2010; Lara; Rostagno, 2013). Reductions in food supply and quality may impact consumers globally but will especially impact low-income consumers – up to about 183 million people could go hungry under projected climate change scenarios (Mbow et al., 2019).

Responding to current and future climate change scenarios requires two possible and necessary approaches, mitigation and adaptation. Applying better agricultural practices and developing more adapted and tolerant varieties to this new climate reality are essential and urgent for the sustainable

increase of agricultural production in the coming decades. Increased tolerance to high temperatures and long periods of water restriction are selection criteria that should be applied in breeding programs to develop new cultivars, particularly in the most sensitive phases of the crop development cycle. Biotechnology tools such as molecular markers, gene editing, transgenesis, and microbiome, as well as more accurate large-scale phenotyping techniques, can and should be employed to accelerate the availability of genotypes adapted to specific regional conditions modified by recent climate changes.

A reduction in public funding for breeding programs has been observed worldwide compared to private sector companies, despite the undeniable importance of public research in agricultural production and food security, especially in medium and long-term scenarios (Alson et al., 2009). This decline is accompanied by Intellectual Property (IP) protection practices and increased private investments. This change has been observed mainly in seed companies, which went through a series of acquisitions and mergers, resulting in greater and concentrated participation in the world market and technological domain (Gutiérrez et al., 2014; Ray et al., 2015; OECD, 2018). Until the mid-1990s, the participation of national companies, including Embrapa, in the Brazilian soybean and corn seed markets was 70% and 30% (Silva et al., 2015). However, with the creation of the Patent (1996) and Plant Variety Protection (1997) Laws, biotechnology multinationals massively introduced proprietary seeds with biotechnological traits into the Brazilian market (Castro et al., 2006). As they do not invest in technological development on the same scale, the share of public companies in the seed market was reduced to less than 10% (Silva et al., 2015).

High investments and innovation capacity enable these large multinational companies to continuously develop new cultivars with specific genetic modifications through research and development pipelines integrating improvement and biotechnology. Traits incorporated include herbicide and pest resistance and, more recently, drought tolerance (Eisenstein, 2013; Rippey, 2015). A pipeline is the sequential process of research and development phases in which technologies move, broadly speaking, through discovery, validation, optimization, and commercial launch. As pipelines work in a continuous flow, at any given time, different technologies are in different phases of technological maturation throughout their development. Many of these biotechnological traits are also combined in the same cultivar or even licensed to competing companies. However, as they almost exclusively develop new biotechnology-derived crop traits in the countries of origin where their research and development centers are located, the maximum performance of these technologies is not achieved in global consumer markets where new discoveries are incorporated or adapted to local research and development programs. Therefore, it is strategic for the Brazilian agricultural sector, currently responsible for a quarter of the Gross Domestic Product (GDP), that public and private national institutions strengthen their scientific and technological production to contribute to the national development of appropriate technologies and varieties to our demands.

Embrapa is recognized for its remarkable track record in improving agricultural crops, a wide network of test sites, and qualified multidisciplinary human resources. In response to current demands, the operation of a biotechnology pipeline is emerging in this institution. The objective is to provide new biotechnology research in developing varieties adapted to the new and complex conditions imposed by climate change. This initiative requires long-term funding, highly coordinated approaches, and cross-disciplinary partnerships, including those between public and private companies. Public-private partnerships have been successful in discovering, developing, and commercializing biotechnological traits. The multinationals Bayer, BASF, Corteva, Syngenta, and their respective public and private partners have developed cultivars with increasingly advanced biotechnological traits. In two cases, genes introduced by genetic engineering were able to increase corn grain yields by 15% and 120% under intense water

stress in a wide range of tested sites (Castiglioni et al., 2008; Nuccio et al., 2015). Cultivars of economically important crops generated by gene editing are already entering the North American seed market. In early 2019, soybean cultivars generated by the transcription activator-like effector nucleases (TALEN) system with high oleic acid content were released for commercial use. In addition, in 2019, corn hybrids with high amylopectin content generated through CRISPR-Cas9 were in the pre-release phase, with prospects of being available in 2020 (Kim; Kim, 2019; Gao et al., 2020). Likewise, improvement of drought resistance traits mediated by CRISPR-Cas9 is expected (Shi et al., 2016). In Brazil, similar initiatives to implement agricultural biotechnology pipelines are incipient, both in the public and private sectors, especially those involving the initial phase of discovering new genes of biotechnological importance.

At the end of 2017, a partnership between Embrapa, UNICAMP, and FAPESP created the Genomics for Climate Change Research Center (GCCRC), bringing together the first two institutions in agricultural biotechnology. The Center's mission is to develop biotechnological assets that will increase crop drought and heat tolerance over the next 10 years while transferring the developed technologies to the productive sector. Biotech assets under development can fit into different intellectual protection strategies that balance value and access to technology. These include (but are not limited to) genes, alleles, and gene constructs – which can be appropriately developed into traits by third parties – microbial inoculants, synthetic microorganism communities, new support technologies such as gene expression regulatory methods and elements, and regulatory patent know-how.

The GGCRC is the consolidation and expansion of the Mixed Unit for Research in Genomics Applied to Climate Change (UMiP GenClima), a technical-scientific cooperation agreement between Embrapa and UNICAMP signed at the end of 2012. Researchers and analysts from both institutions compose the GCCRC, where activities follow steps from a pipeline similar to large biotechnology companies, although smaller. National and international partners, public and private, contribute to the GCCRC team to achieve its mission.

In the digital transformation scenario, information and communication technologies can be integrated into biotechnology by adding computational tools in a research pipeline, including sensors and cameras for monitoring and data capture. Furthermore, these data will require mathematical models and statistical analyses in order to process the large volume of data generated by "omics". Altogether, these approaches will provide research advances for plant genetic improvement. The GCCRC contributes to the implementation of digital agriculture in Brazil through biotechnology and molecular biology research in its pre-production phase, allowing the development of new biotechnological assets for agribusiness. In this chapter, the GCCRC research pipeline will be presented, showing the steps involved in generating biotechnology-derived crop traits and illustrating how digital technologies help researchers obtain results.

The GCCRC research pipeline

The main research activities of the GCCRC are carried out through a research and development pipeline in biotechnology, which spans from the discovery phase to the proof-of-concept phase under field conditions (Figure 1). The species chosen as the target of the research work was corn, one of the most important agricultural crops in Brazil and in the world, which has a wide availability of genetic and genomic resources.

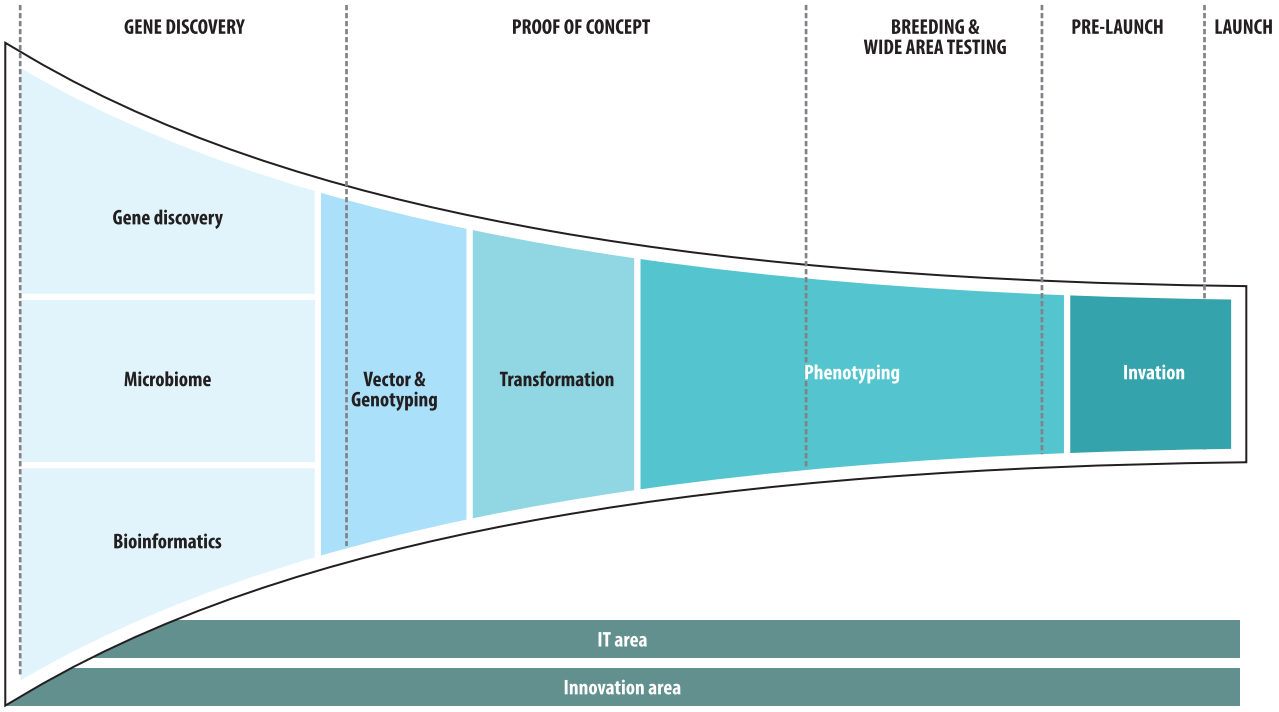


Figure 1. Genomics for Climate Change Research Center search pipeline phases.

The research pipeline has five phases. The first three concentrate most of the efforts of our team:

1) **Discovery:** when new genes and microorganisms are identified and indicated for introduction into the pipeline after the analysis of intellectual property and biosafety.

2) **Proof of concept:** genetic constructs and inoculants are developed, transgenic and edited plants are generated, with the first tests under controlled conditions (growth chambers and greenhouse), and on a small scale in the field. These are carried out for initial assessment of the strategy's effectiveness.

The subsequent phases are carried out in partnership with other organizations by way of collaboration and/or licensing, namely:

3) **Breeding and large-scale testing:** After discovery and selection, these transgenes, edited alleles, and/or inoculants are tested in large-scale field experiments at various locations and at different times. Promising events are being assimilated into elite corn strains.

4) **Pre-release:** development of commercial cultivars containing the technologies.

5) **Launch:** the technologies developed by the center are launched on the agricultural market.

A dedicated infrastructure was built for the effective operation of the research pipeline. The physical structure is composed of a molecular biology laboratory (Figure 2), a plant genetic transformation laboratory (Figure 3), a phenotyping laboratory under controlled environment conditions (under construction), and a modern greenhouse (Figure 4). The molecular biology laboratory houses all the activities of the discovery stage and a large part of the research team (Figure 2). The plant genetic transformation laboratory is equipped with a complete infrastructure for corn transformation, which includes two growth chambers designed for regeneration and acclimatization of transformed plants

(Figure 3). A modern greenhouse was built for growing corn dedicated to embryo production (explants used in genetic transformation), cultivation of transgenic and edited events from the pipeline, generational advancement, introgressions into elite material, inoculant tests, and initial screening experiments in controlled environments. This structure has five environments with temperature control, LED light supplementation, and a screened nursery to accommodate other species under study. All environments have internet access, and environmental conditions are constantly monitored (Figure 4).

Figure 2. Molecular Biology Laboratory. Laboratory entrance (A); meeting room (B); internal view of work benches (C); and office (D).

Figure 3. Laboratory of Genetic Transformation of Plants. External view of the laboratory (A); internal view of the laboratory (B and C); plant regeneration room (D); and plant acclimatization room (E).

Photos: Juliana Erika de Carvalho Teixeira Yassitepe

Figure 4. Vegetation house. Front view of the greenhouse (A); plants growing in the greenhouse and (C); plants growing with supplemental LED lighting (D); exterior night view of the greenhouse (E).

All pipeline routines and processes are registered in a Laboratory Integrated Management System (LIMS), ensuring that information is stored, managed, and tracked correctly, mainly for the purposes of intellectual property, biosafety, integrity of data and procedures (registration of activities, routines, protocols, reports, related documents, etc.).

Discovery of genes and microorganisms

The discovery phase is based on two fronts which focus on the identification and characterization of new (i.e., little or not studied) candidate genes and microorganisms with biotechnological potential to promote increased stress tolerance. Both fronts are structured, to a large extent, in multidisciplinary approaches for exploring the diversity of agricultural and wild plant species. There is special attention given to adaptations for limiting environmental conditions characterized by the incidence of one or more stresses. These approaches demand intensive use of bioinformatics and computational tools due to the large volume of analysis data produced by genomic technologies and related sciences (trancriptomics, metabolomics and metagenomics).

Global climate change, associated with population growth and competition for land will increasingly shift food and bioenergy production to marginal environments (Backlund et al., 2008; Ornella et al., 2012). These environments are characterized by one or more abiotic stresses, such as suboptimal levels of temperature (heat or cold) and water availability (drought or flood), unfavorable soil physical properties, and very low nutrient availability which impose limitations on productivity (Belaid; Morris, 1991). Therefore, the challenge posed by global climate change requires developing new adapted and more productive agricultural genotypes in stress-prone environments which naturally limit plant growth. Thus, understanding the adaptation of plant species to limiting environments and using a series of morphological, physiological, biochemical, and molecular changes in response to stresses that negatively

affect growth and productivity can contribute to global food and bioenergy production in the next decades. Investigating wild species (not only those that are evolutionarily close, but also those distant from cultivated species) provides knowledge to guide the development of new genotypes capable of thriving in marginal environments (Mccouch et al., 2013). Among these species are the extremophiles and those tolerant to desiccation.

Extremophile organisms inhabit severely limiting environments, such as those characterized by extremes of temperature, water or nutrient availability and high salinity, stresses that occur alone or simultaneously (Oh et al., 2012). In turn, desiccation-tolerant species can survive long and/or severe periods of drought, supporting dramatically low levels of relative water content in vegetative tissues (Bartels; Hussain, 2011). Data sets and approaches derived from "omics" are a growing resource for the discovery of new genetic characters, where biotechnological use can contribute to abiotic stress adaptations. Many of these characters are unique to individual species (or a small group of related species) or belong to gene families present in many plant species that are functionally diversified through duplication and adaptive selection (Gollery et al., 2006; Horan et al., 2008). In such perspective, genes of unknown function represent 20-40% of the genes in each new sequenced genome, the majority constituting species-specific differences (Gollery et al., 2006, 2007), and are potentially associated with adaptive mechanisms, including stress tolerance (Mittler; Blumwald, 2010).

The Velloziaceae family of angiosperms contains the most desiccation tolerant species (approximately 200 of its 270 species). More than 80% of species in this family occur in South America, where the greatest morphological diversity is also found. The largest genus, *Vellozia*, comprises both tolerant and sensitive species to desiccation, offering an excellent model to study the evolution of desiccation and drought tolerance characters. *V. nivea* and *V. intermedia*, respectively tolerant and desiccation-sensitive species, are both drought-tolerant, endemic to Brazilian rupestrian grasslands, and highly adapted to its extreme conditions. These environments are characterized by a prolonged dry season, typically between late autumn and early spring, high solar radiation, and rocky, shallow soils which are poor in nutrients, particularly phosphorus. Unlike most model plant species, which originate from environments where nitrogen is the main limiting nutrient, the genus *Vellozia* evolved in an environment where phosphorus is the most limiting nutrient, making it a valuable model for crops grown in tropical soils, where the very low availability of this mineral prevails. The group has been exploring *V. nivea* and *V. intermedia* genomes, transcriptomes and metabolomes, in addition to other species of the same family. The resulting knowledge will help to identify genes and pathways underlying the adaptation of these species to their limiting environments, generating future agricultural genotypes with increased production capacity in marginal environments.

Plant survival under stressful conditions involves a combination of adaptive mechanisms that go beyond the unique contribution of their genomes (Rodriguez et al., 2008; Lau; Lennon, 2012). Microorganisms associated with plant tissues play a role in the adaptation to biotic and abiotic stresses, and play a key role in plant phenotypic plasticity (Woodward et al., 2012; Coleman-Derr; Tringe, 2014). Furthermore, recent advances have shown the existence of an unexplored microbial community with a significant impact on their hosts (Bulgarelli et al., 2013; Souza et al., 2016). These findings make microbiome research an important source of genetic and biological resources for biotechnological use in improving plant adaptation to stressful conditions.

Traditionally, research on microorganisms associated with plants is based on culture-dependent techniques, which are based on the isolation and cultivation of microorganisms. However, the restricted use of these culture methodologies can bias the microbiota sampling, since only microorganisms capable of growing in the culture media are recorded. Furthermore, these methodologies do not

provide information about the real abundance or real functional contribution of an isolate in its original habitat. More recently, large-scale sequencing tools have allowed access to microbial diversity in a crop-independent manner, enabling more accurate mapping of the phylogenetic and functional microbiota profile associated with plants. However, although sequencing techniques elucidate vital issues, the isolation of microorganisms is still necessary for biotechnological applications. Despite being complementary, however, both strategies are rarely used together.

Differently from traditional approaches, the microbiome investigation pipeline makes use of dependent and independent cultivation techniques concomitantly. The use of genomic investigation tools provides information about diversity, colonization patterns and functions performed by the microbiota in association with the plant. These data enable us to identify the most efficient microorganisms in association with plants that promote plant growth. Based on this information, synthetic microbial communities are designed with the collection of isolated microorganisms (Armanhi et al., 2018). Synthetic communities are validated in inoculation experiments to assess their ability to increase stress tolerance and maintain plant productivity even under unfavorable conditions.

In a complementary and synergistic approach to the exploration of plant species in rupestrian fields, this approach has been applied in the pipeline to investigate the strategies by which microorganisms contribute to plant survival in the stressful conditions of these habitats. This is based on the assumption that microbial communities associated with plant species that evolved in environments historically exposed to drought and nutritional scarcity are more likely to promote tolerance to these stresses in the plant than microorganisms originating in environments where such resources are not limiting (Rodriguez et al., 2008; Redman et al., 2011; Lau; Lennon, 2012). These studies are ongoing and allow mapping the composition, abundance, and diversity of bacterial and fungal communities associated with native plants adapted to limiting environments; creation of a comprehensive collection of microorganisms associated with these species; the investigation of plant and microorganism interactions related to plant growth under stressful conditions; and extensive analysis of microbiome genomes (metagenomes, produced from DNA directly recovered from samples) in search of stress tolerance gene functions.

The microbiomes associated with Velloziaceae and other species from rupestrian fields had not been characterized until recently. A first study carried out by the GCCRC, the Joint Genome Institute (JGI, USA) and partners described the identification of great bacterial and fungal diversity and novelty in the microbiomes of two endemic Velloziaceae that inhabit soil and rock in rupestrian fields in Serra do Cipó (MG) (Camargo et al., 2019). Microbial diversity and abundance in epiphytic (external) and endophytic (internal) compartments of roots, stems, leaves and substrates were evaluated by sequencing molecular markers. The root and substrate metagenomes of each species were also sequenced. The results compose the first microbiome databases associated with endemic Velloziaceae species in rupestrian fields. These findings will significantly support the discovery of new microorganisms and, consequently, the potential for obtaining new inoculants.

Proof of concept

The proof-of-concept stage ranges from the design of the genetic constructs that will be inserted into corn plants for the development of transgenic or edited events, as well as the preparation of microbial inoculants for the testing of developed technologies in controlled environments and on a smaller scale, in the field.

After defining the gene constructs containing candidate genes and regulatory sequences, the Vector Construction and Genotyping teams build the vectors and begin the recombinant DNA molecule validation phase with sequencing and other molecular tools, as shown in Figure 1. Once the validated

gene constructs are made available, the Transformation team is called upon to carry out the genetic transformation of the target species, corn. The Transformation team performs corn transformation using locally optimized protocols and appropriate corn genotypes. The transformation platform is designed to routinely transform immature embryos using weekly gene constructs provided by the Vector Construction and Genotyping teams. The Transformation team is constantly improving corn transformation protocols through established national and international partnerships. Alternatively, for strategies based on candidate genes (or their related genes) that are identified in corn, gene editing methods are being used to carry out specific modifications. This approach has been used in parallel, while aiming to obtain biotechnological assets. Regenerated plants are evaluated for number of copies and expression level, and edited plants are evaluated for the presence of the edited allele by means of sequencing. Once the genetic transformation or editing is confirmed, the plants are considered transgenic and edited events, respectively, and are later transferred to the greenhouse for crossing (or self-fertilization) and phenotyping by the Phenotyping team.

Microorganisms with a potential role in plant tolerance to abiotic stresses discovered by the Discovery team are organized into synthetic microbial communities. Inoculants with different microorganism combinations and microbial communities are prepared and used in experiments, validating their effectiveness. This is carried out by the Phenotyping team based on the assessment of their ability to promote tolerance to abiotic conditions.

The phenotypic evaluation of plants is one of the most important phases in any cultivar development program, as it will define which genotypes will be eliminated and which will proceed to the next steps. In biotechnology pipelines, this phase is even more important, especially for characters that tolerate abiotic stresses, such as water and heat stress. These are complex features, and are often the effect of the transgene, edited allele, and/or inoculant conferring tolerance. These may be significant, but difficult to separate from the effect of the plant's genetic background. In a pipeline where hundreds of transgenes, edited alleles, and/or inoculants, and thousands of plants must be evaluated, a fast and reliable selection procedure is necessary in order to eliminate unpromising discoveries. The instruments conventionally and routinely used in laboratories to assess plant physiological condition are reliable, but often require destructive sampling, in addition to only allowing specific assessments. Appropriate instruments for continuous and real-time phenotypic assessment allow for a more detailed evaluation of plant physiological responses to environmental variables and treatments. They can also provide additional information with the potential to improve the understanding of the phenotypic response. Several non-invasive and non-destructive technologies have emerged in the field of plant phenotyping in recent years, including spectroscopy, fluorescence, thermography, and digital image capture. These new technologies are currently being used to increase the quantity, quality and plurality of measured characters and allow the distinction of phenotypic effects with the support of modern statistical analyses.

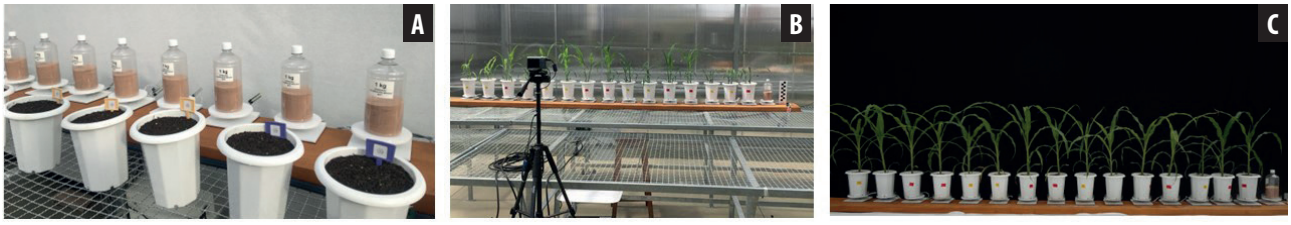
In most biotechnology pipelines, the evaluation of transgenic, edited events and/or microbial inoculants is carried out in three stages: a) initial screening in a growth chamber and/or greenhouse; b) detailed characterization, in a greenhouse; and c) phenotyping in field assays. Controlled environments, such as growth chambers and greenhouses, have a low-cost phenomics platform, which uses sensors and chambers to monitor the environments and plant responses to applied treatments. In the initial screening phase, the plants are evaluated for their stress resistance to short heat and drought cycles during the vegetative stage. Characters are measured in seedling aerial parts and roots according to the expected effect of the event or the tested inoculant. In the detailed characterization phase, plants are evaluated throughout the development cycle, including the reproductive stage and grain production. Various biometric and physiological assessments are performed at different developmental stages, and promising events are

assessed for copy number, expression levels, protein and metabolomic profiles, among others, in order to characterize and understand the effect of the gene/construction and microorganisms applied in the plant.

The low-cost phenomics platform developed at the Center has sensors and cameras that continuously monitor the plants' phenotypic response in real time (Armanhi, 2018). Raspberry Pi and Arduino microcontrollers automatically control sensor readings that monitor the environment (light intensity, relative air humidity, and temperature) and individual plant response (leaf temperature, substrate moisture, and water loss from the pot-plant system). In addition, other parameters can be indirectly obtained, such as the vapor pressure deficit (VPD), a parameter that indicates the plant's propensity to lose water to the environment, and evapotranspiration through loss of water from the pot-plant system. The recorded data is statistically processed, stored, and sent to a local server. An internally developed website allows the graphical visualization of all mentioned parameters in real time.

Microcomputers also automatically control photographic cameras, which record the plants at different angles, at the desired frequency, and send the images to the local server. The entire time image series is accessed remotely, which is used for biometric assessments through available image analysis software. The images can also be used to construct time-lapse image videos, useful in visualizing the continuous response over time, in addition to the observation of small variations throughout the day, such as the expansion and rolling movement of the leaves in response to variations in light intensity and ambient temperature, for example.

Figure 5 illustrates some aspects of the phenomics platform installed in the greenhouse. Scales are used to monitor vessel weight throughout the experiment (Figure 5A). Before the beginning of each experiment, a calibration of the scales is carried out to verify its functioning and the quality of the measurements. A camera is installed to record plant growth in real time (Figure 5B). Cameras and sensors installed in the plants, in the pot and in the environment constantly monitor the weight of the pot-plant system, soil/substrate moisture, leaf temperature, in addition to temperature, relative humidity and ambient light intensity (Figure 5C).



Photos: Jaderson Silveira Leite Armanhi

Figure 5. Some aspects of the phenomics platform installed in the greenhouse. Scales and sensors installed for continuous monitoring of the weight of the pot-plant system and soil/substrate moisture (A); camera installed to continuously record plant growth (B); corn plants monitored continuously in the experiments carried out (C).

After the evaluation phases in controlled environments, promising events and inoculants are selected based on increased resistance to drought and heat stresses (e.g., higher growth rate, lower leaf temperature, lower water loss, higher photosynthetic efficiency, among others) compared to control plants. These are subsequently moved on to the field evaluation phase.

In the field phenotyping phase, the tested events are evaluated in experiments with water restriction, in the reproductive phase, in at least three environments and two different times. Agronomic characters and grain production are evaluated. Events that demonstrate superiority over controls, in more than one location and time, are selected and proceed to the next steps in the pipeline, where building optimizations, elite germplasm introgression, and large-scale testing are performed.

Enhancement, large-scale testing, pre-release and release

The research pipeline stages after the proof of concept are carried out in partnership with public and private institutions that show interest in advancing the technologies for later commercialization.

After the proof-of-concept phase when compared to control treatments, transgene, edited allele, and/or microbial inoculant showing superior field effect on drought and heat tolerance, can be explored in product development pipelines. Edited genes and alleles can be incorporated into improvement programs as an additional source of variability for tolerance to abiotic stresses. Large-scale tests in various locations and years should be carried out as part of the routine for selecting superior genotypes in improvement programs. These may indicate the potential for gains that the introduction of the edited gene or allele could generate in new cultivars. The team works together with partners to ensure an optimal evaluation of the events developed by the center, following all the required biosafety standards.

Similarly, microbial inoculants that present superior performance in field tests in the proof of concept phase should be investigated in extended tests, mainly to evaluate the effects of genotype x inoculant interaction and performance in different locations and seasons. In addition to agronomic efficacy, the development process of a commercial inoculant involves a series of tests aimed at identifying the best formulation. The dose to be applied, the application and storage conditions, and others follow the recommendations of the Ministry of Agriculture, Livestock and Supply (MAPA).

The innovation team will develop actions for: a) collaboration and prospecting for partnerships; b) technology assessment; c) mapping and monitoring of potential markets for our technologies. All these activities are incorporated into the Strategic Plan, which works as a guideline for the pipeline and composes the technology showcase. At this point, establishing partnerships with private companies can provide information and demands to guide the development of applicable technological solutions, in alignment with market demands, and facilitate the transfer in future businesses.

Technology transfer must consider the design of new commercial models, which justify exploring new varieties of market technologies and/or cultural hybrids. Since the objective is the advancement of technologies to the proof-of-concept phase, some of these models may consider licenses and commercial benefits contemplating investments made by a licensee in regulatory and administration processes, and product development.

Final considerations

The GCCRC has corn crop as a research target, but the developed technologies could potentially be transferred to other agricultural crops. The GCCRC has built a modern infrastructure to meet the pipeline's demands, with new greenhouses and laboratories for plant transformation, molecular biology, bioinformatics, and phenotyping. The latter, particularly, has technology that incorporates several low-cost, high-precision sensors, and information systems developed locally for the collection of large numbers of phenotypic data in real time. The first scientific and technological results are already being achieved. Unexplored genes and sometimes of unknown function, associated with responses to abiotic stresses, were discovered, and the first ones are in the proof-of-concept phase in corn and in field tests in sugarcane. The team already masters the technology of gene editing in corn, and edited plants are continuously generated. Synthetic microbial communities composed of beneficial microorganisms that increase corn yield under stressful conditions have been discovered and tested under controlled conditions and in the field. Recent efforts in the sequencing and assembly of genomes and microbiomes

of plants in rupestrian fields open a new path to be explored, in search of new genes and microorganisms adapted to water and nutritionally limiting environments. Following the pipeline rationale, new genes and microorganisms are continually being discovered and tested.

The research carried out within the GCCRC leverages the digital transformation in agriculture, thus promoting the development of new cultivars with genetic modifications that incorporate tolerance to drought and other stresses, thus contributing to the country's ability to sustainably grow and saving natural resources.

References

ALSON J. M.; BEDDOW, J. M.; PARDEY, P. G. **Agricultural research, productivity, and food prices in the long run. Science**, v. 325, n. 5945, p. 1209-1210, Sept. 2009. DOI: 10.1126/science.1170451.

ARMANHI, J. S. L. **Construção de uma comunidade sintética bacteriana promotora do crescimento vegetal oriunda do microbioma de cana-de-açúcar**. 2018. 148 f. Tese (Doutorado em Genética e Biologia Molecular) – Universidade Estadual de Campinas, Instituto de Biologia, Campinas.

ARMANHI, J. S. L.; SOUZA, R. S. C.; DAMASCENO, N. B.; ARAÚJO, L. M.; IMPERIAL, J.; ARRUDA, P. A Community-based culture collection for targeting novel plant growth-promoting bacteria from the sugarcane microbiome. **Frontiers in Plant Science**, v. 8, article 2191, 2018. DOI: 10.3389/fpls.2017.02191.

BACKLUND, P.; JANETOS, A.; SCHIMEL, D. **The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States**: synthesis and assessment product: report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. Washington, DC: Environmental Protection Agency, Climate Change Science Program, 2008. 240 p.

BARTELS, D.; HUSSAIN, S. Resurrection plants: physiology and molecular biology. In: LÜTTGE, U.; BECK, E.; BARTELS, D. (ed.). **Plant desiccation tolerance**. Berlin: Springer, 2011. p. 339-364. (Ecological studies. Analysis and synthesis, v. 215). DOI: 10.1007/978-3-642-19106-0_16.

BELAID, A.; MORRIS, M. L. **Wheat and barley production in rainfed marginal environments of West Asia and North Africa**: problems and prospects. Mexico, D.F.: CIMMYT, 1991. (CIMMYT economics working paper 91/02).

BULGARELLI, D.; SCHLAEPPI, K.; SPAEPEN, S; VAN THEMAAT E. V. L.; SCHULZE-LEFERT, P. Structure and functions of the bacterial microbiota of plants. **Annual Review in Plant Biology**, v. 64, p. 807-838, 2013. DOI: 10.1146/annurev-arplant-050312-120106.

CAMARGO, A. P.; SOUZA, R. S. C.; DE BRITTO COSTA, P.; GERHARDT, I. R.; DANTE, R. A.; TEODORO, G. S.; ABRAHAO, A.; LAMBERS, H.; CARAZZOLLE, M. F.; HUNTEMANN, M.; CLUM, A.; FOSTER, B.; FOSTER, B.; ROUX, S.; PALANIAPPAN, K.; VARGHESE, N.; MUKHERJEE, S.; REDDY, T. B. K.; DAUM, C.; COPELAND, A.; CHEN, I. A.; IVANOVA, N. N.; KYRPIDES, N. C.; PENNACCHIO, C.; ELOE-FADROSH, E. A.; ARRUDA, P.; OLIVEIRA, R. S. Microbiomes of Velloziaceae from phosphorus-impoverished soils of the campos rupestres, a biodiversity hotspot. **Scientific Data**, v. 6, article number 140, p. 1-11, 2019. DOI: 10.1038/s41597-019-0141-3.

CASTIGLIONI, P.; WARNER, D.; BENSEN, R.J.; ANSTROM, D. C.; HARRISON, J.; STOECKER, M.; ABAD, M.; KUMAR, G.; SLAVADOR, S.; D'ORDINE, R.; NAVARRO, S.; BACK, S.; FERNANDES, M.; TARGOLLI, J.; DASGUPTA, S.; BONIN, C.; LUETHY, M. H.; HEARD, J. E. Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. **Plant Physiology**, v. 147, p. 446-455, 2008. DOI: 10.1104/pp.108.118828.

CASTRO, A. M. G. de; LIMA, S. M. V.; LOPES, M. A.; MACHADO, M. dos S.; MARTINS, M. A. G. **O futuro do melhoramento genético vegetal no Brasil**: impactos da biotecnologia e dos direitos de proteção do conhecimento. Brasília, DF: Embrapa Informação Tecnológica: Embrapa-Secretaria de Pesquisa e Desenvolvimento, 2006. 506 p.

COLEMAN-DERR, D.; TRINGE, S. G. Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. **Frontiers in Microbiology**, v. 5, article 283, 2014. DOI: 10.3389/fmicb.2014.00283.

CONCENTRATION in seed markets: potential effects and policy responses. Paris: OECD Publishing, 2018. 236 p. DOI: 10.1787/9789264308367-en.

DAMATTA, F. M.; GRANDIS, A.; ARENQUE, C. C.; BUCKERIDGE, M. S. Impacts of climate changes on crop physiology and food quality. **Food Research International**, v. 43, n. 7, p. 1814-1823, Aug 2010. DOI: 10.1016/j.foodres.2009.11.001.

EISENSTEIN, M. Discovery in a dry spell. **Nature**, v. 501, p. S7-S9, Sept 2013. DOI: 10.1038/501S7a.

GAO, H.; GADLLAGE, M. J.; LATIFFE, H. R.; LENDERTS, B.; YANG, M.; SCHRODER, M.; FARRELL, H.; SNOPEK, K.; PETERSON, D.; FEIGENBUTZ, L.; JONES, S.; CLAIR, G. S.; RAHE, M.; SANYOUR-DOYEL, N.; PENG, C.; WANG, L.; YOUNG, J. K.; BEATTY, M.; DAHLKE, B.; HAZEBROEK, J.; GREENE, T. W.; CIGAN, A. M.; CHILCOAT, N. D.; MEELEY, R. B. Superior field performance of waxy corn engineered using CRISPR-Cas9. **Nature Biotechnology**, v. 38, p. 579-581, 2020. DOI: 10.1038/s41587-020-0444-0.

GOLLERY, M.; HARPER, J.; CUSHMAN, J.; MITTLER, T.; GIRKE, T.; ZHU, J. K.; BAILEY-SERRES, J.; MITTLER, R. What makes species unique? The contribution of proteins with obscure features. **Genome Biology**, v. 7, article R57, 2006. DOI: 10.1186/gb-2006-7-7-r57.

GOLLERY, M.; HARPER, J.; CUSHMAN, J.; MITTLER, T.; MITTLER, R. POFs: what we don't know can hurt us. **Trends in Plant Science**, v. 12, n. 11, p. 492-496, Nov 2007. DOI: 10.1016/j.tplants.2007.08.018.

GUTIÉRREZ, A. P.; ENGLE, N. E.; DE NYS, E.; MOLEJÓN, C.; MARTINS, E. S. Drought preparedness in Brazil. **Weather and Climate Extremes**, v. 3, p. 95-106, June 2014. DOI: 10.1016/j.wace.2013.12.001.

HORAN, K.; JANG., C.; BAILEY-SERRES, J.; MITTLER, R.; SHELTON, C.; HARPER, JF.; ZHU, J. K.; CUSHMAN, J. C.; GOLLERY, M.; GIRKE, T. Annotating genes of known and unknown function by large-scale coexpression analysis. **Plant Physiology**, v. 147, p. 41-57, 2008. DOI: 10.1104/pp.108.117366.

IIZUMI, T.; RAMANKUTTY, N. Changes in yield variability of major crops for 1981–2010 explained by climate change. **Environmental Research Letters**, v. 11, n. 3, article 034003, 2016. DOI: 10.1088/1748-9326/11/3/034003.

KIM, J.; KIM, J. New era of precision plant breeding using genome editing. **Plant Biotechnology Reports**, v. 13, p. 419-421, 2019. DOI: 10.1007/s11816-019-00581-w.

LARA, L.; ROSTAGNO, M. Impact of heat stress on poultry production. **Animals**, v. 3, p. 356-369, 2013. DOI: 10.3390/ani3020356.

LAU, J. A.; LENNON, J. T. Rapid responses of soil microorganisms improve plant fitness in novel environments. **Proceedings of the National Academy of Science of the United States of America**, v. 109, p. 14058-14062, 2012. DOI: 10.1073/pnas.1202319109.

MBOW, C. C.; ROSENZWEIG, L. G.; BARIONI, T. G.; BENTON, M.; HERRERO, M.; KRISHNAPILLAI, E.; LIWENGA, P.; PRADHAN, M. G.; RIVERA-FERRE, T.; SAPKOTA, F. N.; TUBIELLO, Y. XU. Food security. In: SHUKLA, P. R.; SKEA, J.; BUENDIA, E. C.; MASSON-DELMOTTE, V.; PÖRTNER, H. O.; ROBERTS, D. C.; ZHAI, P.; SLADE, R.; CONNORS, S.; VAN DIEMEN, R.; FERRAT, M.; HAUGHEY, E.; LUZ, S.; NEOGI, S.; PATHAK, M.; PETZOLD, J.; PEREIRA, J. P.; VYAS, P.; HUNTLEY, E.; KISSICK, K.; BELKACEMI, M.; MALLEY, J. (ed.). **Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems**. [S.l.]: Intergovernmental Panel on Climate Change, 2019. p. 437-550.

MCCOUCH, S.; BAUTE, G. J.; BRADEEN, J.; BRAMEL, P.; BRETTING, P. K.; BUCKLER, E.; BURKE, J. M.; CHAREST, D. C.; CLOUTIER, S.; COLE, G.; DEMPEWOLF, H.; DINGKUHN, M.; FEUILLET, C.; GEPTS, P.; GRATTAPAGLIA, D.; GUARINO, L.; JACKSON, S.; KNAPP, S.; LANGRIDGE, P.; LAWTON-RAUH, A.; LIJUA, Q.; LUSTY, C.; MICHAEL, T.; MYLES, S.; NAITO, K.; NELSON, R. L.; PONTAROLLO, R.; RICHARDS, C. M.; RIESEBERG, L.; ROSS-IBARRA, J.; ROUNSLEY, S.; HAMILTON, R. S.; SCHURR, U.; STEIN, N.; TOMOOKA, N.; KNAAP, E. VAN DER; TASSEL, D. VAN; TOLL, J.; VALLS, J.; VARSHNEY, R. K.; WARD, J.; WAUGH, R.; WENZL, P.; ZAMIR, D. Feeding the future. **Nature**, v. 499, p. 23-24, July 2013. DOI: 10.1038/499023a.

MITTLER, R.; BLUMWALD, E. Genetic engineering for modern agriculture: challenges and perspectives. **Annual Review of Plant Biology**, v. 61, p. 443-462, 2010. DOI: 10.1146/annurev-arplant-042809-112116.

NUCCIO, M. L.; WU, J.; MOWERS, R.; ZHOU, H. P.; MEGHJI, M.; PRIMAVESI, L. F.; PAUL, M. J.; CHEN, X.; GAO, Y.; HAQUE, E.; BASUL, S. S.; LAGRIMINI, L. M. Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. **Nature Biotechnology**, v. 33, p. 862-869, 2015. DOI: 10.1038/nbt.3277.

OECD. **Concentration in Seed Markets**: Potential Effects and Policy Responses. Paris: OECD Publishing, 2018.

OH, D.-H.; DASSANAYAKE, M.; BOHNERT, H. J.; CHEESEMAN, J. M. **Life at the extreme**: lessons from the genome. Genome Biology, v. 13, article number 2747, 2012. DOI: 10.1186/gb4003.

ORNELLA, L.; CERVIGNI, G.; TAPIA, E. Applications of machine learning for maize breeding for stress. In: VENKATESWARLU, B.; SHANKER, A. K.; SHANKER, C.; MAHESWARI, M. (ed.). **Crop stress and its management**: perspectives and strategies. Netherlands: Springer Netherlands, 2012. p. 163-192. DOI: 10.1007/978-94-007-2220-0_5.

RAY, D. K.; GERBER, J. S.; MACDONALD, G. K.; WEST, P.C. Climate variation explains a third of global crop yield variability. **Nature Communication**, v. 6, article number 5989, p. 1-9, 2015. DOI: 10.1038/ncomms6989.

REDMAN, R. S.; KIM, Y. O.; WOODWARD, C. J. D.; GREER, C.; ESPINO, L.; DOTY, S. L.; RODRIGUEZ, R. J. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. **Plos One**, v. 6, issue 7, e14823, 2011. DOI: 10.1371/journal.pone.0014823.

RIPPEY, B. R. The U.S. drought of 2012. **Weather and Climate Extremes**, v. 10, part A, p. 57-64, Dec 2015. DOI: 10.1016/j.wace.2015.10.004.

RODRIGUEZ, R. J.; HENSON, J.; VAN VOLKENBURGH, E.; HOY, M.; WRIGHT, L.; BECKWITH, F.; KIM, Y.-O.; REDMAN, R. S. Stress tolerance in plants via habitat-adapted symbiosis. **The ISME Journal**, v. 2, p. 404-416, 2008. DOI: 10.1038/ismej.2007.106.

SHI, J.; GAO, H.; WANG, H.; LAFITTE, W. H.; ARCHIBALD, R. L.; YANG, M.; HAKIMI, S. M.; MO, H.; HABBEN, J. E. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. **Plant Biotechnology Journal**, v. 15, p. 207-216, July 2016. DOI: 10.1111/pbi.12603.

SILVA, F. F.; BRAGA, M. J.; GARCIA, J. C. Concentração nos mercados de sementes de milho, soja e algodão: uma abordagem ECD. **Agroalimentaria**, v. 21, p. 133-150, 2015.

SOUZA, R. S. C.; OKURA, V. K.; ARMANHI, J. S. L.; JORRÍN, B.; LOZANO, N.; DA SILVA, M. J.; GONZÁLEZ-GUERRERO, M.; ARAÚJO, L. M.; VERZA, N. C.; BAGHERI, H. C.; IMPERIAL, J.; ARRUDA, P. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. **Scientific Reports**, v. 6, article number 28774, June 2016. DOI: 10.1038/srep28774.

WOODWARD, C.; HANSEN, L.; BECKWITH, F.; REDMAN, R. S.; RODRIGUEZ, R. J. Symbiogenics: an epigenetic approach to mitigating impacts of climate change on plants. **HortScience**, v. 47, n. 6, p. 699-703, June 2012. DOI: 10.21273/HORTSCI.47.6.699.