

Genomics for Food, Health and Nutrition

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According to IPCC-2014 (<http://www.ipcc.ch/>), by the end of this century, the Earth's average temperature is expected to increase from 2 to 4.5 °C. With the elevation in temperature, the production of major crops will be reduced evidently around the world. The population is supposed to grow to about 9 billion in 2050 and global agricultural production may need to be increased by 100–110% to meet the global food requirements (FAO, 2009; Tilman et al., 2011). The growing demand for food at global scale and the increasingly harsh and challenging environmental conditions due to climate change constitute serious threats to Agriculture. Adaptation strategies to combat Climate Change must be developed particularly in developing countries where Climate Change is threatening for the food security of millions of people.

The AdaptNET project, an Erasmus+ project funded by the European Commission, fits into this context and is born with the aim of improving the professional competences in the areas of Climate Change Management (CCM) and Climate Smart Crop Development (CSCD) in southeast Asia. The project wants to achieve it by exchanging information and building capacities between higher education institutions of the EU and India.

Recently, in a webinar organized by the AdaptNET partner International Crops Research Institute for the Semi-arid Tropics (ICRISAT) (<http://cegsb.icrisat.org/vii-nggibci/>), some key elements in the Development of Climate Smart Crops were discussed. Two of the talks from the webinar “Genomics for Food, Health and Nutrition” will be reported here.

The first talk: “**Breeding crops to feed 10 billion**” given by Dr. **Lee Hickey**, pointed out the importance of speeding up the process of breeding, when the necessity of improving yield productivity is becoming more and more urgent due to the increasing global population and harsher environmental conditions. He focused on a new method, called “speed breeding” developed by his team. The method can speed up the breeding process, allowing to grow up to 6 generations in one year (Watson et al., 2018). Dr. Hickey is currently working as a plant breeder and crop geneticist, within the Queensland Alliance for Agriculture and Food Innovation at The University of Queensland, Australia.

The second talk that is reported here, “**Elucidating the genetic architecture of pre-anthesis drought tolerance in barley by invasive and non-invasive phenotyping**”, was given by Dr. **Kerstin Neumann**, a scientist at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. She presented the possibility of using non-invasive phenotyping in barley to identify QTLs related to abiotic stresses, such as drought.

Breeding crops to feed 10 billion

Dr. Lee Hickey's vision on speed breeding (SB) was interestingly illustrated by a video that introduced the involvement of Queensland university in the development of the technology. SB is an useful method that allows to obtain mature viable seeds for many species in a shorter time, by modifying the conditions in which the plants are grown. Speeding up the breeding process is fundamental in the current situation, where the increasing population and the climate change are demanding for more productive varieties that can grow under stressful conditions, such as drought and heat.

The Queensland researchers developed the idea of speeding up the breeding process while looking at NASA experiments in the 1980s, aimed at growing wheat in space. These experiments used controlled temperature and constant light to increase the growth speed. The University of Queensland in 2003 coined the term “speed breeding” referring to a method that they used to hasten wheat breeding. Nowadays the SB protocols are in very high demand: in a period of 12 months, the Hickey’s team received more than 100 requests for SB protocols from breeders and scientists from 24 countries around the globe.

At Queensland University, they developed SB protocols for many species, for instance, 6 generations per year can be achieved for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), and pea (*Pisum sativum*) and 4 generations for canola (*Brassica napus*), instead of the 2-3 which are obtained under normal glasshouse conditions.

The elevated number of species for which SB protocols have been demonstrated to be effective suggests that a SB protocol could possibly be developed for any crop. In light of that, some key elements need to be considered for developing a SB protocol for the species of interest, most importantly the temperature and light requirements (Hickey et al., 2019). Even though the majority of SB protocols has been developed for long-day species, the controlled modification of the photoperiod applied to amaranth (*Amaranthus spp.* L.) has been shown to result in overall shorter generation time (Stetter et al., 2016). This shows that modifying the conditions in a species-specific manner can lead to the development of SB protocols even for short-day flowering plants. In the Nature Protocols paper recently published by Ghosh et al., 2018., the Authors describe the possibility of building a bench-top cabinet that allows to design SB protocols on a small scale with around 1000 USD. The cited paper also describes all the hardware components and the set-up conditions of LED-supplemented glasshouse for SB, presenting the existing structures at Queensland University and at John Innes Centre (Ghosh et al., 2018). It was also discussed the possibility of increasing the density of plants/m² to reduce the cost per single plant in SB program. The authors demonstrated that it is possible to grow up to 1000 plants/m² of barley using the SB protocol, obtaining between 6-12 seeds per plant, that is enough for single seed descent in breeding programs.

The SB method is rapidly being acquired by many laboratories worldwide and many results have been obtained in the very recent past. Facilities have been built by companies and research institutes in America, India, Ethiopia, Lebanon for applying SB to wheat, pearl millet, rice, chickpea. An important project, named “AVISA” in collaboration with ICRISAT has the objective of establishing SB facilities to accelerate breeding pipelines for crops important for Asia and Africa: sorghum, millets, groundnut, chickpea, pigeonpea. During this project, SB protocols for sorghum and pearl millet have been developed and allow to grow up to 4 generations per year. Furthermore, it is possible to increase the density of plants/m² also for these crops, and to grow up to 600 sorghum plants/m² under SB conditions. A recently developed SB protocol for pigeonpea allows to obtain 4 generations/year (Saxena et al., 2019). Very recently, it was also developed a new protocol for soybean, rice and amaranth that reduces the generation time using 10h day of photoperiod and using specific wavelength for each species (Jähne et al., 2020)

Building a SB facilities can be expensive and not many research institutes can afford it. However, the idea of “speed breeding capsule”, that represents a small facility equipped with the cooling and lighting system needed for SB of “orphan crops”, can be implemented by using dismissed refrigerated containers, ideally powered by solar panels reducing the costs and making this resource more accessible (Chiurugwi et al., 2019). Stuart Kemp, from Victoria in Australia, actually built a SB capsule of 15 m², with the capacity of growing 15000 chickpea inbred lines.

Dr. Hickey then presented the numerous applications of the SB method. The breeding programs aim at the introgression of useful genes that are usually coming from wild accessions. To obtain an elite variety containing these traits, many generations of backcrossing, followed by crosses for genes stacking are required. The use of SB can reduce the time needed for performing a breeding program in the field from 8 to about 2 years, when 4 generations of the crop can be grown per year (Hickey et al., 2019).

Another application of speed breeding resides in accelerating genome editing (GE) pipelines. The GE pipelines requires the cloning of the genes and the availability of tissue culture protocols. As a result, few lines are amenable to gene editing, which in many cases is a lengthy process that requires a specialized laboratory. In the GE pipelines, SB can be used to reduce by 3 months the time required for a standard protocol for barley editing (Watson et al., 2018). Dr. Hickey also presented the very challenging possibility of taking CRISPR-Cas9 out of the lab, to reduce the cost and making the technology more accessible for breeding programs. He showed the theoretical GE method called “ExpressEdit”, where the CRISPR-Cas9 system is directly sprayed on the plants that are growing in SB facilities, exploiting newly developed technology to transfer the editing machinery into the plants. He proposes to use Ribonucleoprotein complexes or clay nanosheets as a delivery system for CRISPR-Cas9 (Hickey et al., 2019; Liang et al., 2017; Mitter et al., 2017). This method has the potential to transform GE from a biotechnology into a highly flexible technology that can be used in breeding programs.

He then pointed out the enormous potential of the integration of genomic selection (GS) and the speed breeding into breeding programs, a breeding system named “SpeedGS”. In a simulation that compares a standard breeding program and the SpeedGS, the genetic gain after 30 years of breeding was 34% higher in the breeding program integrating GS and SB, resulting in a theoretical increase in yield of 0.54 ton/hectare (Voss-Fels et al., 2019). Dr. Hickey then underlined the importance of integrating the SpeedGS within an industrial context. He announced a cooperation between the Queensland University and a local breeding company which resulted in an algorithm that predicts the best crosses to be performed in order to integrate the most desirable traits in a variety in the shorter time possible. Dr. Hickey finally remarked that the SB systems can theoretically be applied to any crop and can play an important role in the reduction of the time and subsequently the costs related to breeding operations and varietal release.

Elucidating the genetic architecture of pre-anthesis drought tolerance in barley by invasive and non-invasive phenotyping

Dr. Neumann introduced the Climate Change issue, focusing on the German situation. She pointed out that a constant increase in mean temperature has been observed in the last 50 years, but more importantly for agriculture, in the last 20 years it has been registered an increase of days/year when the temperature was over 30°C (data from IPK weather station). Interestingly, the last 2 years (2018/2019) were some of the warmest years recorded in Germany and the 2 years with the worst drought condition in many regions of Germany. 2018 was the warmest year since 1881 and the driest year ever recorded in Germany (UFZ Drought Monitor Germany). The environmental conditions registered had a direct effect on the grain yield production in Germany, that was reduced to levels comparable to 2011. It is thus evident the need of developing crops more tolerant to drought stress in Germany. IPK has developed a strategy for the identification of genetic regions linked to the drought tolerance in barley.

Three main phenotyping platforms are available at IPK, that have been developed to collect phenotypic data in the greenhouse, in field conditions and in a plant growth cabinets, that allow to accurately simulate the field growing conditions: <https://www.ipk-gatersleben.de/en/phenotyping/>. At the Leibniz institute, there are many ongoing projects on different crops that uses the highly technological phenotyping facilities.

Dr. Neumann focused on the work described in 2 recent publications (Dhanagond et al., 2019; Neumann et al., 2017), that used the facility for plant phenotyping in the greenhouse. This facility can analyze 520 plants simultaneously, is equipped with an imaging chamber that captures images using different light spectra (VIS, PAM, NIR, Fluo), and controls the watering in a completely automated manner. The images are analyzed using the Integrated Analysis Platform developed by Klukas (Klukas et al., 2014), that combines the different pictures and a series of phenotypic data can be extracted (plant height-width, digital biomass, compactness, color information, etc.). Numerous data are collected through the phenotyping facility and the analysis can be computationally challenging (Chen et al., 2014).

The barley collection used, comprises 100 spring barley accessions, described in (Neumann et al., 2017) and selected from a larger collection broadly used in research (Alqudah et al., 2014; Pasam et al., 2012). In the experimental set-up, each accession was grown in normal and in stressed conditions for 58 days. The drought stress phase lasted 18 days and started at 27 DAS, simulating a drought condition starting in the pre-anthesis phase and followed by a recovery phase. A total of 3 experiments for each treatment with 5 plants per genotype were performed.

The main parameter considered during this analysis was the digital biomass, very strictly correlated to the real biomass (Chen et al., 2018). The biomass measured over time for the plants

grown in normal conditions, allowed the generation of a model that shows the growth curve, from which the Authors extracted an inflexion point that represents the moment of maximum growth. The inflexion point is highly inheritable (0.8) and there is a high range of variability of 9 days between all the genotypes that reflect the flowering time. Two QTLs related to the inflexion point were identified, these QTLs are found on the chromosomes 1H and 7H and are located in the corresponding regions where 2 genes known to be involved in flowering are mapping: 1H “HvHeading” (Afsharyan et al., 2020) and 7H “HvFT1” (Loscos et al., 2014).

When the plants are grown under drought condition, the growth curve, obtained using the digital biomass, changes substantially: after the stress is applied, the curve assumes a bell shape and when the watering is applied for optimal growth, the plant recovers with a relative increase in biomass. The point where the bell shape curve reaches its maximum, identifies the wilting time, that has been considered a representative trait in drought tolerant accessions. The wilting time has been used to define 2 traits:

- The fAW (fraction of available water) at wilting time;
- The average wilting time.

The fAW shows a heritability value of 0.68 and is highly variable: some plants start wilting at a fAW of 25.9% whereas others wilt with a lower fAW of 15.2%. The average wilting time is instead a more heritable trait (0.79) and ranges from 35.4 to 41.7 DAS. Interestingly, the Authors also found a correlation between the wilting time and a decrease in the photosynthetic efficiency. They identified the QTLs related to drought tolerance in the wilting phase, where the photosynthetic efficiency decreased.

Furthermore, they noticed that the tillering was affected by the drought stress. During the wilting phase, the drought reduces the number of tillers. However, in the recovery phase the tillering was generally higher in the drought condition but most of the tillers would ultimately be unproductive. This phenomenon has led them to analyze the possible correlation between flowering time and biomass accumulation. The conclusion is that genotypes with late flowering accumulate more biomass that disfavors them when the drought stress is applied, but they recover better.

Another interesting finding is that the biomass negatively correlates with wilting time, which means that the accessions accumulating a higher biomass in the early stages will more probably wilt earlier. Dr. Neumann then pointed out that accessions originated from countries like Syria and Kyrgyzstan that very often experience drought, showed a higher capacity of tolerating the drought stress. Compared to other accessions, coming from cooler places like Canada and Russia, the landraces from Syria and Kyrgyzstan started to wilt later even if the biomass was the same. A very similar observation was done on wheat cultivars.

Through the analysis of the biomass accumulation over time, 4 QTLs were found to be correlated with the wilting time, however 2 of them were also responsible for the biomass accumulation, due to the strong correlation observed with the wilting time. Using the phenotyping platform, the Authors were also able to identify 7 QTLs responsible for biomass accumulation in normal conditions. 3 of these QTLs are responsible for early biomass accumulation and 4 of them for the biomass accumulation in later stages of development. The 7 QTLs together can explain 44-52% of the genetic variability observed (Neumann et al., 2017).

The analysis of the phenotypic data regarding biomass accumulation under drought condition allowed the Neumann's team to identify a total of 12 QTLs responsible for biomass accumulation under stressed condition. 6 of the QTLs were detected exclusively during the drought stress and 5 QTLs during the recovery phase, while one QTL was associated with biomass in both phases. When they compared the phenotypic effects of these QTLs between normal and stressed conditions, they identified a total of 5 QTLs responsible for drought tolerance; 4 of them are responsible for biomass accumulation during the stress phase, whereas 1 of them is responsible for biomass recovery after the drought condition is removed (Dhanagond et al., 2019).

Later, they performed a fine phenotypic analysis on a subset of the barley collection (25n), measuring different yield parameters: tiller number, straw biomass, grain yield. They concluded that all yield parameters were reduced by the drought stress, namely the average grain yield was reduced by 22%, ranging from 14% to 46%, depending on the genotype.

The work presented by Neumann shows that that growth and abiotic stresses tolerance are highly polygenic and influenced by the environment. Precision phenotyping, if performed in controlled conditions, allows to resolve dynamics of genetic architecture and explores trait relationships during plant cycle. Modelling of growth curves and trait integration reveals drought-related traits useful to classify genotypes according to pre-anthesis drought tolerance.

Conclusions

During this webinar, innovative technologies have been discussed, the application of which can have an important impact on the development of new crops tolerant to abiotic stresses that preserve high yields even under climate challenging conditions. The use of non-destructive phenotyping facilities and its integration with genomic information could be instrumental in the identification of the genetic information needed to generate drought tolerant cultivars, not only in barley, but also in other cereal species. Speed breeding system could be possibly applied either during the phenotyping phase, speeding up the identification of important QTL for abiotic stress tolerance, and during the breeding programs that aim at the introgression of these traits in elite varieties.

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The webinar was organized on 14 May 2020, receiving overwhelming participation, with 3388 registrations from 68 countries. The report was prepared by Angelo Gaiti, AdaptNET project collaborator at University of Milan (UMIL) with the contribution of Prof. Carlo Pozzi, Associate Professor and AdaptNET PI at UMIL and Nilesh Mishra, Senior Scientific Officer, at CEGSB, ICRISAT. July 13th 2020.

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