Towards Next-generation Rice

– Following the Footprints of Late Academician YUAN Longping

Editor's note: "Father of Hybrid Rice", late academician YUAN Longping, left us in May. His dedication and passion to breed China's super hybrid rice has been passed down to the next-generation researchers. To see into the future of next-generation rice, YAN Fusheng, *BCAS* staff reporter, has the honor to invite a group of leading scientists in the field to share with us their own studies and how these studies will implicate and inspire the next-generation rice.

At the end of 1970, LI Bihu, then working as an assistant of late academician YUAN Longping, Member of the Chinese Academy of Engineering, discovered a wild "male sterile" rice plant nearby a ditch from the Nanhong Farm in Sanya, a city located in the south end of Hainan Island of China. This valuable plant, also known as "YeBai" named by YUAN Longping, marks a game-changer in the history of hybrid rice breeding, laying an important foundation for practicing the "three-line" system in hybrid rice breeding. Rice, as a self-pollinating crop, usually resists cross-pollination to activate hybrid vigor in nature; nor is it practical to perform emasculation to spikelets one by one to produce hybrid seeds. Based on this very plant and its descendants, YUAN and his team together with other breeders spearheaded a great wonder in China – feeding nearly one-fifth of the world's population with only about 7 percent of the world's arable land.

As a nation's great loss, YUAN Longping, honored as the "father of hybrid rice", died of organ failure at 91 on May 22, 2021, leaving behind his passion to breed new super hybrid rice – which, as a legacy for the later generations of researchers, will keep going on.

There is no doubt that hybrid breeding has played an irreplaceable role in addressing the global issue of food security. However, other than this technique, one might wonder what techniques or research fields may be able to usher in a new wave of agricultural revolution, which in return would enable us to feed the ever-growing population in the light of global climate changes?

To see into the future of the next-generation rice, *BCAS* invites several leading scientists from the Chinese Academy of Sciences (CAS) and the Chinese Agricultural Academy of Sciences (CAAS) to share with us their own studies, and explore how these studies will implicate and inspire the next-generation rice.

They are invited to share their thoughts on a variety of topics, including what roles are played by root microbiome, how to bring elite alleles lost during domestication back into modern rice cultivars, the options to combine multiple fine traits and breed all-in-one elite crops, how to find a way to keep seeds of hybrid rice and spare the annual acts of seed-making for hybrids, what are the potential of genome editing and enhanced photosynthetic efficiency for the next-generation of hybrid rice, and finally the stories of sea rice and giant rice – the leading roles in YUAN's dreams.

Plants Fond of Farming Some Root Microbes

Dr. BAI Yang

Researcher from the State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology (IGDB), The Innovative Academy of Seed Design, Chinese Academy of Sciences, who takes a favor in studying how a plant interacts with its root microbial community for soil improvement and sustainable agriculture.

***BCAS:** Why did you choose to study root microbiome?*

BAI Yang: The study of root microbiome, or the root-associated microbial communities that inhabit around plant roots is a newly emerging topic, and also a good frontier to explore. More importantly, these root microbes are shown to influence plant growth and health.

***BCAS:** What is the relationship between root microbes and the plant host? And how can the know-how drawn from such studies help improve crop traits, such as in the case of rice?*

BAI Yang: Root microbes and the plant host are, normally, mutually beneficial: plants provide nutrients, such as carbon source, for root microbes; while the microbes help plants adapting to soil environment.

Studies have shown that root microbes can improve the plant's nutrients uptake, disease resistance, and tolerance to environmental stress, such as soil salinity and drought. More interestingly, plants are found to use a palette of mechanisms to shape the composition of root microbes to their own benefits – it is like, plants are "farming their own root microbes" to confront distinct environmental conditions or adapt to environmental changes. On the other hand, via exerting influence on the plant's nutrient absorption, disease prevention and stress tolerance, the root microbes can also, to some extent, shape the rice traits. Hence, it is a new promising direction, though a challenging one, to integrate root microbes with a matched rice genotype to maximize the benefits in a setting-specific manner. Taking root microbes into the picture of modern rice breeding may help feeding the increasing human population and confronting the effects of climate change.

Bring Back the Lost Elite Alleles

Dr. CHU Chengcai

Researcher from the State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology (IGDB), the Innovative Academy of Seed Design, Chinese Academy of Sciences. Dr. CHU's research interests mainly pertain to mining rice genomes for elite alleles that contribute to fine traits in rice, and using them for rice breeding.

BCAS: *Your recent study published in Nature, entitled "Genomic basis of geographical adaptation to soil nitrogen in rice", shows that nitrogen-use efficiency of wild rice is higher than that of modern rice cultivars. What do you reckon would be the reason for that? Could this, the loss of certain good traits, be a general phenomenon during crop domestication and modern breeding?*

CHU Chengcai: Characterized by the cultivation and promotion of dwarf varieties, the "Green revolution" that began in the 1960s has greatly increased global food production. Its main driving force is the large-scale use of fertilizers, particularly the nitrogen fertilizers. Dwarf varieties are not sensitive to nitrogen fertilizers, which protects them from lodging and yield reduction as results from excessive use of fertilizers. Modern breeding has been mostly conducted under high nitrogen supplies, which could be a probable cause to the loss of genes related to efficient nitrogen utilization. On the other side, due to the prevailing overuse of fertilizers, farmers tend to plant fertilizer-tolerant varieties (featured with low nitrogen-use efficiency). All these factors combined contribute to the prevalence of low nitrogen-use efficiency among modern cultivars, including rice.

BCAS: *What is its implication in breeding future rice with improved nitrogen-use efficiency through bringing this lost allele back from their wild cousins or poor-soil landraces? And what are you planning to do in this regard?*

CHU Chengcai: Our finding shows that the exclusive pursuit of high yield in modern crop breeding has led to a great loss of genetic diversity, while landraces still retain a lot of good traits found in the wild varieties by contrast. Therefore, we can improve modern rice varieties by bringing the lost allele back from landraces that largely preserve the valuable genes of wild rice. By doing so, we may be able to usher a new wave of green revolution by producing environment-friendly crop plants with less fertilizer input but high yield.

Our team has brought genes of high nitrogen-use efficiency back into several modern rice varieties. The improved rice varieties achieved the same yield with 20–30% reduced nitrogen supply. Notably, three of them yield 9.2 to 11.7 tons per hectare at 44% cut on nitrogen fertilizer use – the average yield of China's rice per hectare is about 7 tons at normal nitrogen supply. These results demonstrate the feasibility and great potential of breeding crops with improved nitrogen-use efficiency. These rice varieties with high nitrogen-use efficiency have been recognized as the first batch of "green super rice" by the leading experts of the National High Technology Research and Development Program. Our team will keep mining valuable genes from the pool of landraces and wild germplasms, expecting to find the keys to the problems we are facing in sustainable agriculture.

Creating an "All-in-one" Elite Rice Variety

Dr. FU Xiangdong

Researcher from the CAS Institute of Genetics and Developmental Biology (IGDB) with his research focused on elucidating the molecular mechanisms of how different agronomic traits interact with each other, and simultaneously improving multiple fine traits to create all-in-one elite crops through the method of molecular design breeding.

BCAS: *Your team has pinned down many new genes related to high yield and quality in rice, as well as genes related to high nitrogen-use efficiency. Assuming you seek to combine these beneficial traits, or elite alleles (i.e., gene variants located at the same locus in the genome), into one rice variety, what would you do to make it happen? Where the real challenge lies?*

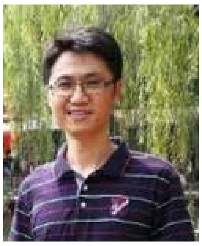
FU Xiangdong: These agronomic traits, as you mentioned, are controlled by multiple genes and influenced by the environment. It is difficult to simultaneously improve them through conventional breeding methods. Alternatively, our team seeks to first identify the key genes regulating traits of interest, as well as the allelic variants. We are also trying to understand how these different traits interact with each other. Our final goal is to create all-in-one elite crops that contain all fine traits of interest by design breeding.

We have practiced it to our course. For example, after confirming four genes, *GW8*, *GW7*, *dep1* and *NGR5*, that collaboratively control rice yield, grain quality and nitrogen-use efficiency, we first sought to acquire the distribution of their allelic variations among the modern high-yield rice cultivars. We then grouped different elite alleles of these genes into rice to check for the best combination. In Cooperation with breeders, we finally designed new state-approved elite rice varieties, namely the '*ZhongHeYou*-1' and '*WuKeJing*-210'.

Enabled by powerful whole-genome sequencing and whole genome selection breeding techniques, it is not so hard to gather multiple fine traits into one crop variety. The real difficulty lies in how to select the right combination of different elite alleles that performs best in boosting the chosen traits. For the existence of 'gene-gene' and 'gene-environment' interactions, the same design scheme that tends to converge chosen fine traits, when applied to varieties with different genetic backgrounds or planted in different ecological areas, may turn out differently or even opposingly.

In another practice, we tried to aggregate five genes that regulate grain shape and weight to simultaneously improve rice yield and quality. We found that rather than the simple full combination of 5 elite alleles, a combination of 3 elite alleles and 2 non-elite alleles instead produces the best result. Therefore, we reckon, it is important to figure out how different traits interact with each other and design the optimal combination of elite alleles by integrating artificial intelligence with field experiments to breed all-in-one elite crops.

From "Three" to "One": Sparing the Year-by-year Seed-making for Hybrid Rice



Dr. WANG Kejian

Researcher at the State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences. Dr. WANG's research focuses on fixing the hybrid vigor in rice by genome editing, and has made a breakthrough in 2019. His work may make Mr. YUAN Longping's dream of the 'one-line' hybrid system really come true, which earned him the 2020 Tan Kah Kee Young Scientist Award.

BCAS: *You were awarded the "2020 Tan Kah Kee Young Scientist Awards" for your achievement in clonal reproduction of hybrid rice through seeds. What kind of problem does the research address?*

WANG Kejian: It is mainly aimed at the challenge of "you can't keep seeds from the hybrids" faced by the hybrid rice industry. Hybrid rice has significant 'heterosis', or 'hybrid vigor' as we commonly known, but the hybrid vigor gets lost in its offspring. As a result, the application of hybrid rice highly depends on the year-by-year production of hybrid seeds. The seed-making is very laborious. It consumes huge amounts of lands, human labors and material resources. All these adds up the cost of hybrid seeds, which is much higher than that of conventional seeds. This problem also severely restricts the further development and global promotion of hybrid rice.

And our job is to deal with this challenge. We have been seeking to find a new way to permanently fix hybrid vigor and spare the trouble of making hybrid seeds annually, which in return would greatly reduce the price of seeds and ensure food security.

BCAS: *It was reported that Prof. YUAN Longping called you and asked about details as soon as he was informed about this breakthrough. What is his long-term expectation of your work? How is this research going so far? Would you share with us the latest progress? What is your next plan?*

WANG Kejian: Prof. YUAN was very pleased. He commented that this work proves the feasibility of apomictic reproduction in hybrid rice, and is really a breakthrough in the field of apomictic reproduction. He also urged my team to keep advancing in this direction, and improve the apomictic reproduction further so that it can someday make the 'one-line' hybrid system really come true and apply it in rice production.

Though our current work proves the feasibility of apomictic reproduction in hybrid rice, the plants that grown from the clonal seeds of hybrids can only yield a limited number of seeds. In this regard, we have been working on how to increase the number of seeds, and currently have obtained many rice plants that grow more seeds. Based on these materials, we will seek to mine the key genes related to seed number and converge them to make the plant grow enough seeds to suffice practical application, in other words, making the 'one-line' hybrid really come true. We also hope to see the success of fixing hybrid vigor in rice being extended to other crops.

We also want to build a technical platform that serves to cultivate apomictic reproduction crops to greatly reduce the cost of making hybrid seeds, so that hybrid seeds will cease to be the barrier for agricultural production.

Harnessing Genome Editing for Crop Improvement

Dr. GAO Caixia

Researcher at the Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences. Dr. GAO is a pioneering scientist in applying various genome editing techniques in plants for crop improvement.

BCAS: *You have done a lot of pioneering work applying genome editing tools in rice and other crop plants. What do you think about the potential role of plant genome editing in rice breeding? What kinds of problems could be solved by genome editing, which could be much harder to solve with other techniques?*

GAO Caixia: Hybrid breeding has been widely used in crop breeding and agricultural production for its effectiveness in boosting crop yield. There are still many problems remained to be solved before its wider applications, such as the creation of male sterile line and reproductive barrier existing in many hybrid plants. Genome editing, as a newly emerged power, may provide solutions to these problems – for example, simple acts of gene-knockout could create male sterile line in many plants – and bring hybrid breeding to another level.

As early as 1987, Prof. YUAN Longping proposed a three-step pathway – from "three-line" to "two-line", and finally to the "one-line" hybrid system – with gradually reduced complexity and enhanced efficiency along the step-wise course. Led by Prof. YUAN, the "three-line" and the "two-line" systems had been successfully achieved in the 1970s and 1980s. However, the "one-line" hybrid system, which relies on clonal reproduction of hybrid rice through seeds to fix hybrid vigor, lagged far behind expectations, mainly due to the huge difficulty in deciphering the underlying mechanisms of apomixis, a type of asexual reproduction, as well as the limited ability of conventional breeding technologies in this regard.

The emergence of genome editing technology is changing the game. In 2019, a Chinese group and an American group respectively used genome editing to substitute mitosis for meiosis in rice. By knocking out three key meiotic genes, *REC8*, *PAIR1*, and *OSD1*, the two groups each independently developed asexual propagation lines. Further, either by simultaneously activating *BBM1* in the egg cell or by knocking out *MTL*, the two groups each established an apomictic reproductive system in rice and thereby obtained clonal seeds from hybrid rice, turning Prof. YUAN's dream of the "one-line" system into reality. Although this "one-line" system still needs further improvement before practical application, it evidently demonstrates the great power of tuning genome editing for breeding technology innovation.

BCAS: *Could you elaborate a little bit on directed evolution of plant genes in vivo? How different is it from the conventional directed evolution conducted in vitro or in bacterial or yeast cells?*

GAO Caixia: The *in vivo* directed evolution of plant genes is a technique of applying genome-editing tools to mutageniz e chosen genes to create an enormous library of gene mutants, and using them to screen for desired traits of interest via high-throughput phenotype screening and genotype analysis. Therefore, it represents a new breeding technique to quickly produce crops with new or improved traits.

Compared with the traditional *in vitro* directed evolution, *in vivo* directed evolution based on genome editing have many advantages. For example, it can work on broader types of functional genes, and it does not require the transferring of evolved genes back into the plant to verify whether it works.

BCAS: *Your group has reported a herbicide-*

resistant trait in rice resulting from in vivo directed evolution. Can this technology be used to improve other agronomic traits, such as rice yield, grain quality and, tolerance of abiotic stress like soil salinity, drought, and extreme temperatures? Where does the challenge lie?

GAO Caixia: In addition to herbicide-resistant genes, the technique is also applicable to functional genes related to various agronomic traits, including crop yield, shoot architecture, quality, disease- and insect-resistance, and nutrient absorption, which are otherwise almost impossible to approach using traditional directed evolution based on bacterial or yeast screening. To directly evolve the functional genes of agronomic traits as we mentioned above, preparing the library of mutants is the easy part. The real difficulty lies in building high-throughput analyzers for phenotype analyses in a trait-specific manner, to which the newly emerged research field called "plant phenomics" may lend a hand.

Boosting the Photosynthetic Efficiency in Crop Plants

Dr. ZHU Xinguang

Researcher at the National Key Laboratory for Plant Molecular Genetics, Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences. Dr. ZHU's research focuses on identifying new options to improve photosynthetic light use efficiency of plants, which can be used to support breeding high-yielding crops with high photosynthetic energy conversion efficiency. Dr. ZHU's research has led to discovery of new genes controlling photosynthetic efficiency of crops under different light environments, such as high, low, and fluctuating light conditions.

BCAS: *It was reported that Prof. YUAN Longping personally invited you to work with his State Key Laboratory of Hybrid Rice. After joining the Lab, you launched a research team to work on photosynthesis. Could you share with us what your team seeks to solve? And what is its implication in crop improvement, such as rice?*

ZHU Xinguang: The question that I like to address is how to improve the efficiency of photosynthesis, because improving photosynthetic efficiency, especially the efficiency of canopy photosynthesis, is regarded as a major option to dramatically improve crop yield potential in the next phase of hybrid rice breeding. The yield potential of hybrid rice has been steadily improved in the last few decades, largely attributed to the effective utilization of the hybrid vigor and ideotype. So far, we have developed both the measurement facility and theoretical analysis tools to study canopy photosynthesis. These will be combined with the recent advances in genomic sequencing technologies to mine molecular markers that control canopy photosynthetic efficiency. These molecular markers then could be incorporated into the future rice breeding programs to improve canopy photosynthesis for greater yield potential. This same approach could also be used to improve the yield potential of other major crops.

Sea Rice and Smart Agriculture

Dr. LI Jiming
Deputy Director of the Qingdao Sea Rice Research and Development Center, dedicated to the development and the promotion of sea rice that can be planted in saline-alkali soils to feed people and meanwhile improve the soils.

BCAS: *You have worked with Prof. YUAN Longping in breeding and promoting sea rice. What was his expectation for sea rice?*

LI Jiming: Prof. YUAN had high expectations of saline-alkali tolerant rice, commonly known as sea rice. He hoped that we could promote sea rice over the country and plant it in about 6.67 million hectares of lands in 8 to 10 years, which would produce an extra yield of 30 billion kilograms of rice and feed an extra 80 million people.

BCAS: *What have you achieved and what is your next plan?*

LI Jiming: In 2017, we started regional tests of saline-alkali tolerant rice. As of 2020, four indica-type sea rice varieties got state-approval, and hence were ready for production and promotion. Meanwhile, we conducted many trials for its industrialization in combination with the country's saline-alkali soil improvement. As a result, the nationwide planting area of sea rice in saline-alkali soils increased to 6,000 hectares in 2020, and this number is expected to increase by 10-fold by 2021. As for the upcoming year of 2022, so far, the contracted planting area has mounted to 400,000 hectares.

As for our future plan, we will look closer into the physiological and genetic mechanisms that enable sea rice to grow under high saline-alkali conditions, and breed more new sea rice varieties with higher tolerance to saline-alkali conditions through molecular design and mutation breeding techniques. We will also redouble our efforts in sea rice promotion. Based on the National Saline-Alkali Tolerant Rice Technology Innovation Center, we plan to plant 67 to 130 million hectares of sea rice. Moreover, we are also hoping to see Prof. YUAN's sea rice hybrids to be planted in other countries with abundant saline-alkali soils. Finally, we also seek to advance further on the "smart agriculture" for sea rice. By integrating various information technologies such as 5G technology and AI, we are looking forward to bringing the sea rice breeding, planting, processing and distribution, and brand building to a new level.

Giant Rice and "Economy under Rice Leaf"

Dr. XIA Xinjie
Researcher at the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Dr. XIA is renowned for breeding the giant rice, which is featured with huge biomass, high yield, and versatile ability of adapting to various environments. Moreover, they are good for integrated ecological planting-and-cultivation.

***BACS:** You have bred a new rice variety that grows into giant plants. What are the advantages of the giant rice?*

XIA Xinjie: The advantages of giant rice over ordinary varieties can be seen in eight aspects. First, it is both high yield and high quality. Its theoretical yield can reach over 15,000 kilograms per hectare in a single season. Meanwhile, it tastes very good – high-yield rice usually does not taste so good as a contrast. The straws of giant rice also have economic values. They can be used as raw materials for silage production, papermaking and building materials because of their high content of cellulose. The tall, huge and dense canopies of giant rice can provide shade and habitat for frogs and other aquatic life, making the paddies an ideal environment for integrated ecological planting and cultivation. Giving its potential a full play can substantially increase farmers' income. Our giant rice can grow in the intertidal zone, or the mud flats, because it can tolerate saline and alkali and withstand ups and downs of the tides; it can even stand the repeated socking by seawater – they literally merit the name of "sea rice". Its strong roots and stumps can increase the organic content of the soils, which is good for soil improvement.

The paddies of giant rice also have the potential for ecotourism. Image that you are taking a small boat or a bamboo raft into the paddies where the giant rice has grown into shape: the reed-like leaves swinging in the breeze, the golden ears of rice hanging high, the fishes and frogs swimming in the water – such a nice view is definitely worthy of a good visit. The planting of giant rice also brings great ecological benefits. Their huge biomass can absorb carbon from the atmosphere and reduce carbon emissions, contributing to the global effort towards carbon neutrality.

***BCAS:** Which climatic conditions or areas can the giant rice adapt to?*

XIA Xinjie: Giant rice plants are robust, with strong roots and stems, and can adapt to a variety of climactic conditions and growing environments. Notably, they can grow well in barren lands and saline-alkali soils. For example, giant rice planted on a beach in Guangdong province withstood tides and seawater soaking, and produced 7,800 kilograms of rice per hectare in 2020, together with 7,500 kilograms of greasyback shrimps cultivated in the same paddy fields. Interestingly, we find that giant rice irrigated with alternative seawater and freshwater produces a better taste.

The strong roots of giant rice plants allow them to grow in drought soils, and their huge size makes it possible to grow them in water where fish is cultivated at the same time. For example, we conducted the integrated planting and cultivation in a town of Sichuan province in 2020 in about 26 hectares of lands, and harvested about 7,500 kilograms of rice per hectare together with 9,000 kilograms of fish per hectare on average.

Currently, giant rice varieties are mainly adapted to the southern rice-planting regions, with the northernmost planting areas reaching Tianjin and parts of Shandong. Giant rice varieties suitable for cultivation in the vast northern rice-planting regions are still under development.

***BCAS:** Have they already been planted by farmers?*

XIA Xinjie: Giant rice is still experiencing a stage of demonstrative planting. The planting of giant rice is mainly combined with cultivation of various economic animals, such as frogs, ducks, shrimps and fishes. We name this practice combining rice planting and cultivation as the "economy under rice leaf". Large-scale planting of giant rice for merely high-yield rice production is still on trial.

***BCAS:** What is your next plan and your expectations of giant rice hold for the future?*

XIA Xinjie: We will further improve the current giant rice varieties and breed the next-generation giant rice with higher yield and better quality. Through hybridizing with rice male sterile lines from various regions, we strive to make new elite hybrids of giant rice capable of growing under different regional and climatic conditions, meeting the demands of both southern and northern rice-planting regions in China. We will also further promote ecological planting-and-cultivation model, or as we call it the "economy under rice leaf", to greatly improve the outputs of rice paddies, as well as ecological benefits such as carbon absorption and carbon sequestration. Meanwhile, we also seek to explore the options to integrate ecological planting-and-cultivation model with "smart agriculture" by building the Internet of Things, sensors and monitoring, and smart field management system. We also need to locate the key genes related to the "huge" traits, and figure out the underlying mechanism of giant rice. This new knowledge would in return help us breed new varieties of giant rice with improved traits.

The breeding of giant rice varieties represents a breakthrough in the history of rice development, and is the best way to enable the next-generation rice to become high-yielding or ultra-high-yielding. Further improvement of giant rice and their promotion to the whole country would surely make our rice production leap to a new level, which is of great significance to our country's food security. Relaxing in the rice shades will no longer be an unreachable dream, and 15,000 kilograms rice yield per hectare will become a common scene. After the successful domestic promotion and cultivation, we are looking forward to seeing the giant rice grow in Southeast Asia, and in other parts of the world, to benefit the all mankind.

The superior features, such as high yield, high quality and huge shoot architecture, make giant rice very suited for integrated ecological planting and cultivation, or as we call it the "economy under rice leaf", opening a new option for increasing the income of famers. The wide application of this new model of giant rice planting would solve many problems existing in agricultural and rural areas. It points to a way to greatly improve the economic and ecological benefits of rice fields. For example, sparing the use of pesticide and fertilizers in rice planting, as well as the use of antibiotics in culturing economic animals. I think, there could be good chances to make our agriculture more efficient and greener, to rebuild the earth's ecological harmony, and to recover the beauty of nature. Giant rice can also offer an opportunity to revitalize the countryside, and to make the farmers richer, contributing to the vista of "Low-carbon China".