5Gs for climate-smart agriculture

Dr. Rajeev K Varshney, Research Program Director Genetic Gains, ICRISAT and AdaptNET partner was part of a webinar series where the '**Applications of Omics in climate-smart agriculture'** were discussed. Here you can find a report of the talk, during which he described useful technologies and methods that could have significant impacts on accelerating and enhancing the effectiveness of breeding programs, specifically focused on the development of improved varieties tolerant to abiotic stresses and resistant to pests.

In his presentation, named '5Gs for Climate Smart Agriculture', he correlated crop improvement technology to other modern technologies. He underlined that mobile technology evolved incredibly fast in the past few decades, from 1G (1980) to 5G (2020), passing from a navigation speed of 2.4 kbps to 10 Gbps. He strongly believes that a similar acceleration in crop improvement technology will be necessary to meet the increasing global food demands and to keep production higher against the vagaries of Climate Change conditions.

His presentation continued by citing the challenges that agriculture is going to cope with; it is expected that, by 2030, because of the changing climatic variations exacerbated by the phenomenon of Global Warming, nine out of the 10 major crops will experience a reduction or a stagnation in their growth rates. For instance, the growth rate of the maize crop will be reduced by 12% with a relative increase in price by 90%. In addition, the world population is expected to reach about 10 billion people by 2050, and India will be the most populous nation with an expected population of 1,66 bn people (from the actual 1,4 bn). The increasing population will require to increase global food production by 70%, according to the UN Department of Economics and Social Affairs. It appears that the agricultural sector will need to increase the global food production with less available input resources: water (whose availability may reduce by 40% by 2050), arable lands and labour.

If we look back to the role of plant breeding in agriculture, it brought many benefits: it helped the farmers to withstand pests and diseases with less inputs, it maximised water, land and nutrients use efficiency and finally it helped stabilizing the yield despite the Climate Change conditions. However, more radical changes and improvements will be necessary to face new challenges. He presented a 5G breeding approach for bringing disruptive changes to crop improvement. Dr. Varshney based his presentation on a recently published paper in Current Opinion in Plant Biotechnology Journal on "5G for crop genetic improvement"[1]

These 5Gs stands for**: Genome, Germplasm, Genes, Genomic breeding (GB), and Gene editing (GE)**

1. **Genome**: The importance of knowing the whole genome sequence emerged for human health interests. In 1990 the world's largest collaborative biological project for the sequencing of human genome did begin, which eventually costed 2 billions US dollars to obtain the genome sequence in 2003 (https://www.genome.gov/human-genome-project). Since 1980, when the 1st technologies for DNA sequencing were set up, the time and money needed for genome sequencing exponentially decreased until today, when it is possible to sequence a new genome with a few hundred dollars using NGS (Next-Generation Sequencing) technologies. In plant science, some major milestones were reached when the Arabidopsis genome and rice genome were obtained, in 2000 and 2002 respectively [2–4]. Dr. Varshney believes that, for efficient crop improvement programs, the genome sequence of the cultivated and related species is required and the advances in genome sequencing technology is making this possible in a short time and at affordable prices. The ICRISAT's Center of Excellence in Genomics & Systems Biology has significantly contributed with the sequencing of the genome of important crops for the Indian subcontinent: Pigeonpea (*Cajanus cajan*), Chickpea (*Cicer arietinum*), Pearl-Millet (*Pennisetum glaucum)*, Groundnut (*Arachis hypogaea*) [5–8]. Having the sequenced genomes of many species is crucial for developing resistant cultivars using the genes of related species; a clear example of this application is the development of a variety of soybean resistant to rust, using a gene derived from pigeonpea [9]. Another possible application presented is the use of the genetic information of pearl millet (which is particularly rich in protective waxes) to improve heat and drought tolerance in other cereal species[5]. Dr. Varshney then presented the concept of **super-pangenome**, which is the development of a pangenome of the pangenomes of different species for a given genus. The super-pangenome provides a complete genomic variation repertoire of a genus and offers precious information and opportunities for crop improvement [10]. Finally, for the genome section, Dr Varshney underlined that knowing the genes sequences is important, but another fundamental information is the temporal and spatial expression of genes. In this context, he cited the recent production of a gene expression atlas through the RNA-sequencing technology of chickpea, pigeonpea and groundnut [11–13].

2. **Germplasm**: the germplasm collections have the potential of including information to be used in crop improvement. Around the world, there are more than 1700 genebanks and Dr. Varshney highlighted the importance of characterizing the germplasm material stored. The characterization needs to be done either at the genomic and phenotypic level and the two levels must be associated. He underlined the effort in genome sequencing of the material stored at ICRISAT and he presented the recent resequencing of more than 400 chickpea genotypes, from wild types to landraces, and breeding lines [14]. Around 5 million SNPs were identified, and these data have been analysed to obtain Genome-wide variations, population structure and the phylogenetic tree of the population. Interestingly, a significant reduction of diversity from wild type genotypes to landraces and breeding lines was observed, suggesting

that about 80% of genetic diversity captured in this study has been lost during chickpea domestication. The genomic data were also used to analyse the center of origin of the chickpea, and the study confirmed the origin to be in the Fertile Crescent/Mediterranean. Most importantly for crop improvement, the genomic data of around 300 chickpea genotypes together with phenotyping data for 20 drought and heat tolerance related traits were collected over several locations and subjected to GWAS analysis in order to identify important agronomic traits: these analyses identified 4 genes that are associated with heat and drought tolerance and could be potentially used in breeding programs or through genetic engineering approaches. In the future, Dr. Varshney believes that sequencing of whole germplasm collections and association with phenotypes should be a primary component for major crop-breeding programs.

3. **Genes**. The genes are the fundamental, physical, and functional units of heredity, and together with other factors, such as the environment, determine the phenotypic traits of an organism. The gene function identification is another pillar of the speaker approach: Many candidate genes for agronomic traits of interest with associated markers have been identified in many crops, using either functional genomic or mapping approaches. However, a large part of these genes is far from being confirmed, and we have very little knowledge of their functioning at the molecular level. Dr. Varshney presented the 'Systems Biology' approach as an effective and comprehensive way to characterize the gene's function. It proposes the possibility of obtaining a full understanding of biological systems by combining-omics approaches- such as genomics, transcriptomics, epigenomics, proteomics, and metabolomics - together with modelling and high-performance computational analysis [15].

Dr Varshney described ICRISAT results in the identification of QTLs and candidate genes for the drought tolerance in Chickpea. Combining the genomic and the phenotypic data from 2 mapping populations, they were able to identify 1 genomic region associated with 12/20 drought tolerance traits that explain 58% of the phenotypic variability observed in the population[16]. This genomic region, named *QTL hotspot*, was then further analysed. A combination of two approaches, namely QTL analysis and gene enrichment analysis, were used to identify candidate genes in the *QTL-hotspot* for drought tolerance. The 1st approach that used recombination bins along with the phenotyping data for 17 drought tolerance related traits, allowed to split the "*QTL-hotspot*" in 2 sub-regions spanning a total of 26 genes, while the 2nd approach, based on significant marker trait associations, showed enrichment for 23 genes. Finally, 4 promising candidate genes having functional implications for drought tolerance in chickpea were validated using quantitative real-time PCR (qRT-PCR).

He then presented an interesting approach used to identify genes that are involved in the leaf rust resistance in groundnut (*Arachis hypogaea*). In this case, to reduce the costs of genotyping by sequencing, he illustrated the possibility to use the QTL-seq approach. QTL-Seq is based on pooling resistant or susceptible genotypes to build two populations that differ

in their susceptibility to the disease. The two populations were then sequenced and the genomic data, crossed with the phenotypic data, allowed the identification of the genomic region associated with the disease resistance. The results obtained strongly suggest the potential of the QTL-seq approach for precise and rapid identification of candidate genomic regions and the development of diagnostic markers for breeding applications[17].

Dr. Varshney then cited how ICRISAT used the System Biology approach to characterise an environment-sensitive male sterile line in pigeonpea. In a recent publication in Plant Genome [18], the authors described how male sterile and fertile anthers from five developmental stages were studied by integrating transcriptomics, proteomics and metabolomics supported by precise phenotyping and scanning electron microscopy. From this analysis, 17 differentially expressed genes in sterile anthers were identified. The study shows that male fertility to sterility transition was mainly due to a perturbation in auxin homeostasis, leading to impaired cell wall modification and sugar transport.

Using the previously described approaches ICRISAT's Center of Excellence in Genomics & Systems Biology together with partners was able to identify between 20 and 50 useful agronomics traits in chickpea, pigeonpea and groundnut.

4. **Genomic breeding (GB)**: this new approach uses data, knowledge, resources, genes and technologies originated by genomic research to enhance breeding programmes [19]. Some examples of the methods used in GB are marker-assisted selection (MAS), marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS). In addition to these GB methodologies, some new approaches such as forward breeding (FB), haplotype-based breeding (HBB) and genomic selection (GS), coupled with speed breeding (SB), have also been suggested for enhancing the precision, efficiency and rate of acquired genetic gain in crop improvement programs [1]. Dr. Varshney is strongly convinced that the GB need to be integrated into most of the crop breeding programs. After this short introduction, he described ICRISAT's success together with partners in developing new varieties of chickpea and groundnut with the genomic breeding approach for the Indian and African agriculture, for instance:

- A drought-tolerant chickpea variety with improved productivity, named "Geletu", was developed with MABC and released in Ethiopia. [20,21].
- Two Fusarium wilt-resistant introgression lines of chickpea were developed and released in India (Super Annigeri 1 and improved JG 74).[22]
- Three popular Indian groundnut varieties were developed for foliar disease resistance and high oleic acid using MABC [23]. This result has been obtained by combining previous studies on groundnut varietal development.[24]

5. **Gene Editing (GE)**: the last but not least breeding tool for importance is the GE technology, which allows making highly specific changes in the DNA sequence of a living organism. Specific DNA modifications can be targeted to DNA genomic sequences using meganucleases: zinc-finger nucleases, TALENs, and Cas9 (and related nucleases). Although these enzymes are structurally different, all can be engineered to recognize and cleave different DNA sequences, allowing to obtain customized modifications. GE has been used to generate useful traits in many crops, such as maize, rice, sugarcane, potato, tomato, grape and many others. The traits introduced using GE include herbicide resistance and disease resistance [25]. For example, Oliva et al. 2019 describe the use of CRISPR/Cas9 technology to generate rice plants that are resistant to the main pathogen that causes rice blight, *Xanthomonas oryzae* pv. *oryzae*. The authors edited the promoters of three rice sucrose transporter genes *SWEET11*, *SWEET13* and *SWEET14* that are required for disease susceptibility. In addition, the team of researchers has developed a diagnostic kit that can trace the disease, its virulence and together with resistance alleles [27]. The GE technologies offer to the crop breeding programs the awesome possibility to customize the plant genomes using the genetic information of every sequenced species. There remains a lack of clarity as to the GMO or non-GMO status of GE germplasm in many countries, limiting the exploitation of this technology[28].

The key takeaway of the lecture was that a comprehensively applied '5G' breeding system can have a key role in the development of climate-resilient, nutritious, high-yielding varieties, while delivering a high rate of genetic gain, including in developing countries where these gains are most needed.

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