INTERFACING CONVENTIONAL AND MODERN APPROACHES TO SPEED UP AND FOCUS PLANT BREEDING

Plant breeding relies on discovering, generating, selecting and utilising genetic variation in agronomic traits. This can be achieved in many ways – and at very different speeds. Our authors present various approaches and technologies – with their strengths and weaknesses – ranging from pollination-based conventional plant breeding to the Crispr/Cas genetic scissors.

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The Crispr/Cas technology – represented here as a model – has already been used to modify traits such as the fruit/grain quality and quantity, nutrient content, bacterial, viral and fungal disease resistance, drought and salinity tolerance and herbicide tolerance.

Photo: Bilderbox.com

Human population grew from one billion to two billion in 123 years, but it grew from five to six billion only in 12 years (1987-1999) and also from six to seven billion in another 12 years (1999-2011). Our population is now predicted to level out at ten billion beyond 2050. Sustaining the more than seven billion people today is already a challenge with the business-as-usual scenario. The increasing numbers of people over time, and recently the growing middle classes, generate an incremental demand on natural resources, which is inconsistent with the natural supply or replenishment of these resources.

The single most important resource for survival is food. Nearly eleven per cent of Earth's surface is devoted to crop production. Yet only 3.5 per cent of it suits crop production without any problems. For the remaining 7.5 per cent, human endeavour through tools and technologies has overcome the problems to make the land good for agriculture. Similarly, making agriculture good and sufficient for humans (including their livestock) has also been possible through constantly improving technologies. One such technology, with a great capacity to feed the world, is plant breeding.

Plant breeding generates new varieties of crop plants that are much more high-yielding than the previous ones. Generating new varieties depends on generating novel or enhanced traits through genetic recombination during cross- or self-pollination (see Glossary on page 10). The efficiency of plant breeding depends on the life-cycle period of the respective crop, which can vary from a few months for grain staples (rice, wheat, maize), through a couple of years (cassava) to many years (perennial fruit trees). Hence pollination-based conventional plant breeding between limited lines undertaken by humans remains a time-consuming technology which cannot keep up with the pace at which crop improvement is required to feed the growing population. Several approaches and technologies have been invented (see Glossary on page 11) in order to hasten the generating and capturing of variations in useful traits such as increased yield or resistance to diseases.

LITTLE PLANT BREEDING GLOSSARY I: BASIC TERMS

Abiotic stress: Environmental stresses such as drought, flooding, heat, cold, salinity, etc. that affect plant productivity.
Allele: Variant form of a gene.
Biotic stress: Stresses that affect plant productivity caused by biotic factors such as microbes, insects, weeds, etc.
Cross-pollination: Refers to pollen cell of one plant fusing with the egg cell of a different plant of the same species to generate the first cell of the next generation.
DNA: Deoxyribonucleic acid (DNA) is the fundamental heredity matter in most living organisms.
Gamete: Haploid cell that fuses with another haploid cell during fertilisation in organisms that sexually reproduce.
Genetic engineering: Refers to direct manipulation of DNA of an organism to bring about a desired change in the phenotype.
Genome: The complete set of genetic material present in a cell.
Genotype: The genetic constitution of an organism.
Heterosis: Heterosis (also **hybrid vigour** or **outbreeding enhancement**) is the improved or increased function of any biological quality in a hybrid offspring.
Hexaploid: An organism with six copies of a haploid set of chromosomes (example: bread wheat).
Introgression: Introduction of a gene/ alleles from one organism to another organism by hybridisation and backcrossing.
Markers: Segments of DNA that can be easily identified to suggest that a particular trait or genomic region is present in a plant making it useful for selecting to take to the next stage.
Molecular marker: Fragment of DNA that is used as a marker to associate with a particular gene/ trait.
Nucleotide: Basic structural unit of DNA.
Phenotype: Observable physical expression of genotype.
Plant genetic resources: Plants of the same species in different eco-geographies exhibit variations in traits in line with adaptations for the particular locale. All such plants taken together form the genetic resource for the species and can be used as donor for introgression of the trait.
Polymerase chain reaction (PCR): A method to amplify specific regions of the DNA in a test tube starting with an extremely small amount of the original DNA coming from any biological source or synthetic chemistry.
Polyploidy: A condition wherein an organism/ cell will have more than two copies of its haploid set of chromosomes.
Recombination: A process by which genetic material between two chromosomes in a cell are exchanged.
Selection: The act of choosing desirable/ adaptable plants in a random population by natural forces or by human interference.
Self-pollination: Refers to a pollen cell of one plant fusing with the egg cell of the same plant to generate the first cell of the next generation.
T-DNA: A piece of its DNA transferred by a bacteria (Agrobacterium) into the plant genome, largely without bias in where it is transferred.
Transgene: Gene introduced into one species from an another species by artificial means.
Transposon: These are genetic elements that can move from one part of the genome to another within a cell.

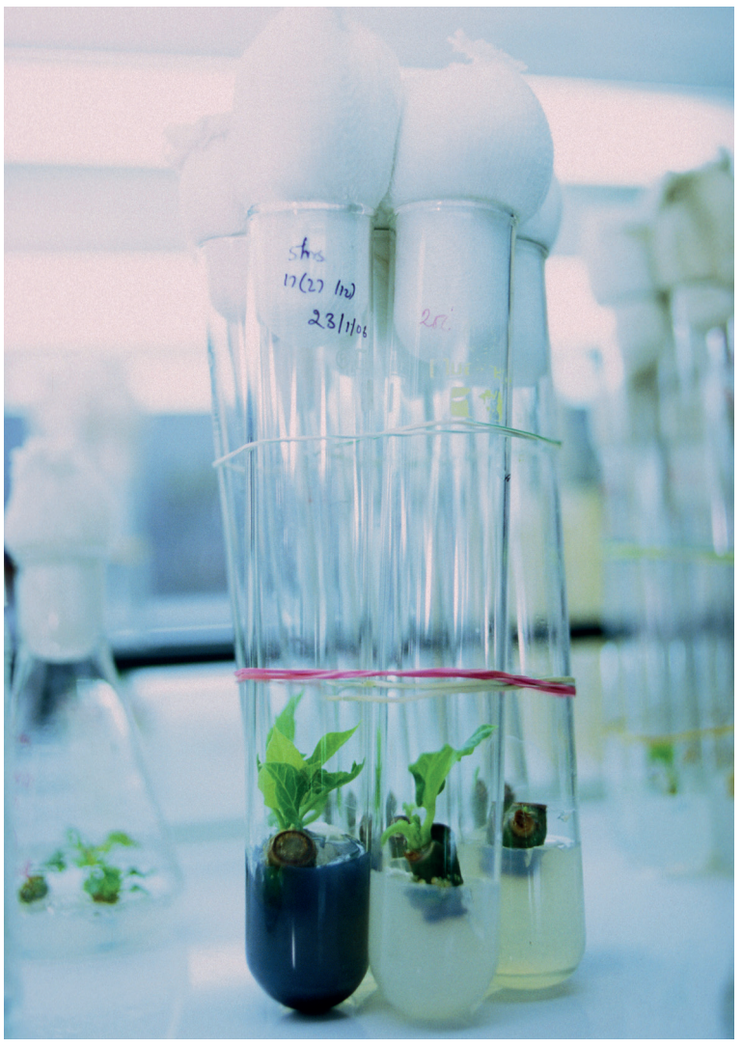
INCREASING PRECISION IN PLANT BREEDING

Plant breeding has been said to be as much an art form as a science. 'Talking to the plant' is the phrase often used by breeders to denote regular visits to the fields to monitor growth, development and reactions to biotic and abiotic factors of a population under study. This allows selecting the desirable plant type from a multitude of plants and propagating it through generations with continuous trait selection, finally leading to a new variety. Such self- or cross-pollination-based conventional plant breeding relied on visual selection of the desired trait. Such a selection would underpin recombination at the genome level. But there was no specific control over the extent of parental gene transfer and genome scrambling. With additional breeding approaches such as backcrossing, recipient genome content could be increased. Molecular markers then allowed tracking the introgression of a trait with DNA while reconstructing most of the recipient genome. This simple method is now reaching the limits of manual handling due to the increasing number of markers, down to single nucleotide polymorphism, and the pace at which they can be scored and analysed.

As an example, marker-assisted selection (MAS) was used for developing "super rice" through already known functional alleles for grain quality and yield using three parents. However, despite the application of automated platform technologies employing numerous markers such as the simple sequence repeat (SSR) markers to perform the functions of DNA extraction, polymerase chain reaction (PCR) and downstream marker scoring and analysis, regions of the recipient genome still remain that are not fully characterised regarding the parent they belong to. To achieve that and to facilitate 'Breeding by Design', the relevant genetic information as well as the tools to use that information need to be in place. The advent of next-generation DNA/genome sequencing technologies is filling some of these technological gaps. Similarly, the technology of double-haploid (DH) plants is very useful in fixing genes in a homozygous state. DH uses in vitro culture techniques to culture haploid single cell pollen grains into diploid plants that then have an exact copy of the original set of chromosomes. The most important resource for plant breeding is the germplasm (available as accessions in respective genebanks for each crop), including the set of wild species, which are not of much use for yield prima-facie but harbour useful genes against biotic and abiotic stresses. These can be harnessed by 'wide hybridisation', which allows mating of a wild and a cultivated accession applying the biotechnological approach of embryo rescue. This enables accessing genetic variation from related species to improve the crops.

An ideal situation would be to maintain the entire genome of the recipient plant and modify it for a single or a few genes – up- or down-regulation of which could affect the trait under consideration. Genetic engineering was a step in that direction. However, it was realised that the process can also generate some changes in the region where the transgene is inserted, not to mention the need for selecting single copy insert events or obtaining marker-free plants. Despite comparatively highly improved precision and stringent regulatory processes that ensure that no unintended effects reach the consumer, this technique has had much opposition in certain regions of the world.

Point mutation through mutation breeding strategies is another approach with a promise of keeping most of the recipient genome intact. Products of mutation breeding do not undergo regulatory procedures and can be certified as organic if grown accordingly. However, it is hardly ever the case that a single mutation occurs in the gene of choice while leaving the rest of the genome unchanged. Agronomic traits are



The efficiency of plant breeding depends on the life-cycle period of the respective crop, which can vary from a few months for grain staples to many years for perennial fruit trees or shrubs, as in the case of jatropha.

Photo: Jörg Böthling

mostly regulated through complex genetic mechanisms. An apparently simple trait depends on many genes. A desirable change noticed through mutation breeding is more likely to occur through multiple changes in different genes. There are however examples of single-base mutation, cloned and characterised as such, leading to improvement in agronomic traits, e.g. the silicon uptake gene in rice.

BIOTECHNOLOGY-MEDIATED GENE/ TRAIT DISCOVERY FOR BREEDING

Progress with innovations in tools and techniques of plant biotechnology combined with ease and speed of experimentation and analysis led to the identification of a number of new genes for agronomic traits such as yield, quality, biotic and abiotic stress resistance. Generating a plant population of individuals with a single gene mutation per plant (saturation mutagenised populations) so that, collectively, potentially all the genes are mutated through T-DNA or transposon insertion in the gene was one manifestation of such tools. However, these results rarely translated into commercial products, mainly because the identified genes did not always have similar qualitative and/ or quantitative effects in other crop plants compared to the model plants (Arabidopsis, tobacco) in which the gene was identified and well characterised. Although rice has also been used as

LITTLE PLANT BREEDING GLOSSARY II: PLANT BREEDING APPROACHES AND TECHNIQUES

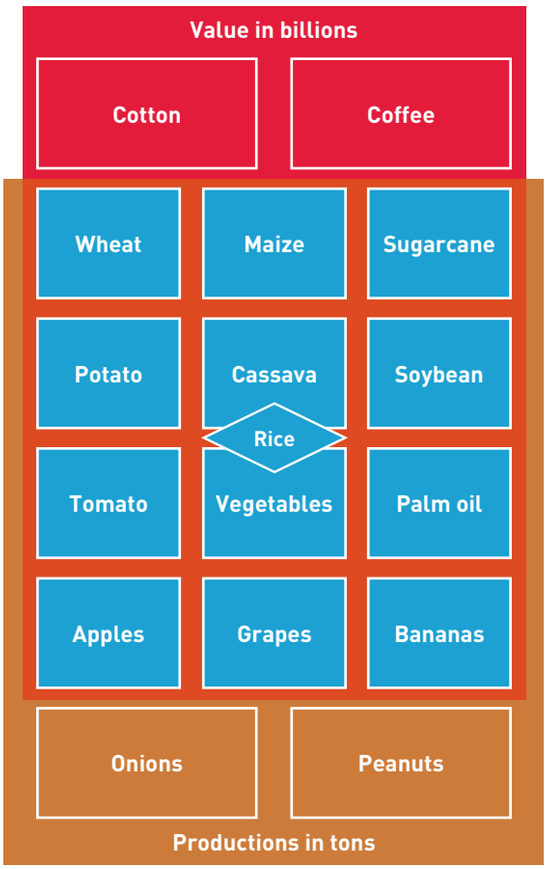
Anther culture: A technique by which plants are regenerated from anthers, the pollen-producing parts of flowers.
Backcrossing: Crossing in which an offspring is mated to one of its parent.
Biotechnology: Molecular markers for traits highly facilitated plant breeding by fast-tracking selection for a trait. Starting from protein isozyme markers, the DNA markers and their sequentially growing speed of analysis up to the single base revolutionised plant breeding, while genetic engineering became a branch for plant breeding in its own right.
Crispr/Cas: A method in which DNA can be cut and changed in a targeted manner. Single DNA building blocks can be inserted, removed or switched off.
Genome Editing: Genetic engineering technology wherein genetic information at specific sites is altered.
Embryo rescue: An in vitro technique to rescue a failing embryo to generate a complete plant.
Epistasis: Genetic interactions between two or more genes affecting a trait.
Heterosis: Plants belonging to the same species but genetically rather diverse exhibit the tendency of improved agronomic traits including yield, quality and resistance to biotic and abiotic stresses. The molecular underpinning of the phenomena is still unclear, but it has been heavily used in maize and rice breeding.
Mutation breeding: Chemical (ethyl methanesulfonate, EMS) and physical (radiations such as gamma rays) agents generate mutations, some of which can be useful and thus harnessed in improving plants.
Polyploidy: Induction of polyploidy can be helpful in wide hybridisations and thus becomes a useful approach in combining traits from parents that would normally not cross-fertilise.
Point mutation: Mutation wherein a single nucleotide change has occurred.
Tissue culture: The first laboratory-based technology for breeding, later subsumed into biotechnology, allowed in vitro culture and regeneration of new plants from different organs through cellular totipotency through which potentially any plant cell can give rise to a full plant.
Wide hybridisation: Crossing of plants may not be limited to individuals within a species. In rare cases, fertilisations between different species of the genera are possible, including wild species, and this increases the breadth of donors for useful traits.

CoMoPheno* breeding: This is the latest approach, whereby high-throughput molecular marker and phenotype analysis supports conventional breeding and the approach capitalises on biotechnology-based molecular physiological understanding of plants for trait discovery and gene discovery, which in turn facilitates the modern GM free breeding approach through the Crispr systems.

* We have used this term coinage and its definition here for the first time as we believe that such interfacing of tools, techniques and approaches holds the future to fast tracking plant breeding by overcoming life-cycle timeline limitations leading to new plant varieties much more quickly. It denotes **Co**nventional **Mo**lecular and high-throughput **Pheno**typing-based breeding. CoMo in Latin means 'bring together', thus the bringing together of the phenotype data on a large scale in much less time for breeding.

a model cereal for such studies and some successes cross over from rice to other cereals, frustratingly, rice genes characterised in one genotype do not always show equal effects in other rice genotypes. This highlights the genetic area of epistasis, which has not been much explored. However, our improved understanding of these genes, and in many cases an improved understanding of physiological processes/ traits based on such genes, now allows us to use that knowledge and information in combination with high-throughput phenotyping, through normal conventional breeding. For example, the need to accelerate breeding can now be addressed through reduction of the generation time by manipulating the photoperiod – the time that a plant is exposed to light within a 24-hour period – with specific light regimes. Such an understanding of the physiological response at the systems level would not have been possible without biotechnology/molecular biology. Similarly, genes for increasing zinc in the rice grain have been identified through the use of molecular and phenotyping techniques, and the genomic regions/ genes will be used to mainstream grain zinc in all rice varieties coming out of the International Rice Research Institute (IRRI).

The world's main food crops



The 13 crops mentioned in blue rectangles are common for globally highest tonnage production and monetary value. With the 13, onions and peanuts make up the top 15 for tonnage production and coffee and cotton make up the top 15 for value. Rice has the highest global value of 337 billion dollars among all crops, while it is third in tonnage.

GENOMIC SELECTION AS A BREEDING TOOL

When marker-assisted selection (MAS) is performed at a very high density, genomic selection (GS) can be carried out. It mainly assesses the presence of a multitude of useful markers and predicts the extent to which a single or multiple traits can be favourably affected by the combination of the markers/ genes in a genotype. The technology is computer intensive, requires a set population and makes genome-wide assessment for predicting phenotypes resulting from biparental or multiparental populations. At IRRI, the plans for low-coverage sequencing of the entire genebank collections of rice accessions will feed the GS models in a very favourable manner for the models to be far more accurate, especially when combined with mechanical and automated phenotyping systems that process experiments and data at a high speed and accuracy (high-throughput phenotyping platforms).

CRISPR/CAS AS THE LATEST TOOL

Instead of random mutations all over the genomes, targeted mutation by Crispr will be of wide value in crop improvement. It can create the targeted mutations in a convenient and quick manner. For example, in hexaploid wheat, three homoalleles could be targeted by a single guide RNA to make the plants resistant to powdery mildew. Similarly for other complex traits controlled by multiple genes, regulatory genes such as transcription factor families could be targeted. Crispr has already been used to modify genes and traits in crops such as tobacco, rice, wheat, maize, tomatoes, cucumber, soybean, potatoes and cassava. Traits such as the fruit/grain quality/quantity, nutrient content (iron, phytic acid, carotenoid) and bacterial, viral and fungal disease resistance, drought and salinity tolerance, yield under drought, potassium deficiency tolerance and herbicide tolerance have been engineered. The ever increasing novel materials and methods for genome editing are bound to be one of the most useful technologies for modifying agronomic traits and thus improving plant breeding. In some countries, the products from this technology have not been placed under the purview of regulatory bodies. With Crispr, it is now easy to expect another green revolution.

FUTURE PROSPECTS WITH CROPS AND BIG DATA

There are 13 common crops in the top 15 crops by global tonnage and global value (see Figure). Along with the 13 common ones, onions and peanuts make up the two for top 15 in tonnage and coffee and cotton make up the two for top 15 in monetary value. There are international agricultural research centres (IARCs) devoted to improving the overall cultivation for seven of these crops (rice, wheat, maize, vegetables, potato, cassava, peanuts), mostly operating under the umbrella of the Consultative Group on International Agricultural Research (CGIAR) system. For the remaining ones, there are other IARCs and national centres. With genomic selection, Crispr and high-throughput phenotyping feeding into the respective big data construct, and set to play a widely critical role in crop improvement, there may be need to have standardised practices and and protocols that can be applicable to most if not all crops. Thus it is opportune to think of an international platform for archiving and disseminating the basic essential knowledge in these areas, along with providing a central hub for advocacy, after due diligence, for acceptance of Crispr-generated plants as the most evolved, precise, harmless, useful and benign technology in the service of humankind under an increasingly malignant climate change scenario.

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