

Democratizing plant biotechnology: The case of seed industry

Dr. Rajeev K Taggar

Taggar RK. Democratizing plant biotechnology: the case of seed industry. *J Microbiol Biotechnol Rep.* 2018;2(1):7-9.

EDITORIAL

A handful of large multi-national seed companies have monopolized the seed industry. The dynamics of business require that these companies exploit only the high business-value crops that are grown over large areas and are consumed by a major chunk of the population (1,2). The result is a world dependent upon a selected few crops, deprived of the benefits of the massive diversity of edible crops, even though there is a wealth of knowledge available on the nutritional and agronomic aspects of a treasure-full of underutilized and neglected crop species. The underutilized and neglected crops are traditionally consumed by people in small localized regions (1). In a world driven by the survival of only the big players, where mergers and acquisitions are commonplace (3), even relatively better-known crops fall by the wayside because these big companies want to invest only for big profits. Same is true for germplasm of even the major food crops. Large seed companies tend to cater to the environment prevalent over large areas of land, thereby ignoring varieties adapted to small localized environmental niches (4). This is where the local seed industry in the developing countries, comprising mainly of the small and medium enterprises (SME), comes into play. In the developing countries, private-sector is the key to delivering quality seeds to the farmers; public-sector is usually not enough (2). The local seed industry has the potential to pick up the crops and the germplasm ignored by the big players, which are nevertheless much adapted to the local environment, cater to the local tastes, are nutritionally rich, empower the local people by way of recognition and enhanced employment opportunities, lead to biodiversity and germplasm conservation by “conservation through use”, bring-in agricultural sustainability in lieu of agricultural over-simplification and could be a major element of food security in the world (1,2,4).

Plant biotechnology is now a major force for driving a successful seed industry in the developing countries (1,2,5). It is required in conjunction with traditional plant breeding to improve the genetic architecture of crop traits, to shorten crop breeding cycle and to develop high-yielding hybrid varieties (4,5). However, biotech is expensive and requires long-term efforts with uncertain results, or at least this is the impression left by some of the biotech undertakings by large seed companies. An example is that of the genetically-modified crops. According to reports by Monsanto (6) and Syngenta (7), the spending for a genetically-modified crop from trait discovery to first commercial sale, including regulatory costs, was at least \$ 136 million. The timeline in both cases was 13 years. The average annual R&D spending of local SME seed companies in India around the same period was \$2 million (8). Democratization of plant biotechnology is the need of the hour.

The major areas of the application of plant biotechnology in SME seed industry in the developing countries are marker-assisted-breeding (MAB), doubled haploids (DH), molecular diagnostics and genetically-modified (GM) crops. While the SME seed industry is self-reliant for applying the first three out of these technologies viz. MAB, DH and molecular diagnostics, they need to partner with the large seed companies, generally the multi-national seed companies, or the academia, to make use of the GM technology. In most cases, SME seed industry gets on licence the GM crop varieties, which have been developed and taken through the regulatory approval by the large seed companies and/or academia. This is followed by inbred line conversion and the development of local company's proprietary hybrids.

The requirements of the seed industry from the molecular marker technologies could be grouped into three categories: i. low marker coverage (<20 markers) for projects like marker-assisted-selection (MAS, foreground) and hybrid seed purity testing ii. medium marker coverage (100-5000 markers) for applications like DNA fingerprinting of inbreds and hybrids, marker-assisted backcross (MABC) and QTL mapping and iii. High to ultra-high marker coverage (>10,000 markers) for projects like genomic selection (GS) and genome wide association studies (GWAS). The SME seed industry requires the marker technologies for low (<20 markers) to medium marker coverage (100-5,000 markers). The applications requiring high to ultra-high marker coverage (>10,000 markers), at this point in time, are either quite expensive or analytically quite demanding.

The SME seed industry has a twin-pronged goal of seeking economy in genotyping and bringing-in in-house self-sufficiency. Among the prominent marker technologies, numerous SNP genotyping platforms have been deployed that use a variety of chemistries, detection methods and reaction formats. These include SNP-arrays, bead-arrays, mass spectrometry based platforms and microfluidics and PCR miniaturization based platforms (9,10). Most of these technologies have been applied quite successfully by the large seed companies for medium to high and ultra-high marker coverage. However, these technologies are not suitable for the applications requiring low marker coverage i.e., <20 markers. Genotyping-by-sequencing (GBS) has been applied successfully for crops with large and complex genomes, empowers by obviating dependence on SNP-arrays/bead-arrays and is cost-effective. However, it is analytically demanding and is suited for applications requiring high to ultra-high marker coverage (11,12). Presently, GBS may not be best choice for SME seed industry. In the coming times, GBS could play a democratizing role for the SME seed industry to undertake projects like GS and GWAS requiring high to ultra-high marker coverage. GoldenGate assay, DArT-array, KASP assay (or KASPar), AmpSeq, rAmpSeq and AmpSeq-SSR have been compared well in the published reports (9,10,13-16). DArT-array and DArT-Seq are suited for medium to high and ultra-high marker coverage but not for low marker coverage. KASP assay is a uni-plex marker technology, suited for low to medium marker coverage (9).

According to Rasheed et al. 2017, while the cost of DArT markers is “moderate”, the cost of AmpSeq markers is “very low” (10). KASPar have been reported to be clearly more cost-efficient than GoldenGate assay (9). AmpSeq/rAmpSeq markers cost less than \$5 for ~3000 datapoints (12,10) while AmpSeq-SSR cost \$ 15 for ~3000 datapoints (14). The minimum stated cost per datapoint for KASPar genotyping services is GBP 0.1 (17) i.e., \$0.133. With in-house assays the price would go down further. At a comparative level, the cost per datapoint using SSR/SNP markers as uni-plex on qPCR/HRM-analyser is about 10 times that figure i.e., ~\$1.300 (personal figures). Gel-based (10) and capillary electrophoresis based marker platforms cannot compete KASPar in cost, efficiency or manpower needs (14). For the SME seed industry, KASP assay using qPCR/HRM-analysis system promises to be an effective and economical option for projects involving low marker coverage while for medium marker coverage, the sequencing-based AmpSeq, rAmpSeq and AmpSeq-SSR markers hold good potential.

DH technology, alone or in combination with the molecular markers, is priceless for shortening the crop breeding time (5). However, *in-vitro* DH

Scientist (Molecular breeding), Green World Genetics Sdn Bhd, Malaysia

Correspondence: Dr. Rajeev K Taggar, Scientist (Molecular Breeding), Green World Genetics Sdn Bhd, Malaysia. email rajeevtaggar@hotmail.com

Received: November 10, 2017, Accepted: November 12, 2017, Published: November 12, 2017



This open-access article is distributed under the terms of the Creative Commons Attribution Non-Commercial License (CC BY-NC) (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits reuse, distribution and reproduction of the article, provided that the original work is properly cited and the reuse is restricted to noncommercial purposes. For commercial reuse, contact reprints@pulsus.com

production protocols are genotype dependent and are sensitive to many conditions like the stage of anther and donor plant (18). Development of DH inducer lines, as in maize (19), and providing access to such resources (20) would be a great democratizing factor for the SME seed industry. Breeding for disease resistance is a perpetual crop improvement goal. So far, pathogen detection has been carried out by the resource-intensive pathogen-culturing, ELISA, pathogen-specific marker assays, immunological strip-tests, microscopy and DNA barcoding. Array-based culture-free approaches like Axiom™ microbiome array for 16S rRNA profiling are also available. Metagenomics, the sequencing-based culture-free analysis of all nucleic acids present in a sample, is coming-up as a resource-efficient and technologically empowering approach (21,22). For the SME seed industry in the developing countries to be successful and to incorporate modern technologies, collaborations and support are required from the public-sector academia, governmental policy making and philanthropic organizations. A dedicated section of the academic curricula needs to be structured according to the actual industry needs, with an eye to the future industry needs, rather than just the publication potential and the promise of remote and uncertain returns. The strength of SME seed industry lies in having a strong crop phenotyping expertise and manpower, which it could offer for collaborations. The academia, government and the philanthropic organizations could contribute with expertise in genetic mapping (GWAS, MAGIC etc.), GBS, genome editing and crop modelling. Also required is the access to germplasm, genomic databases and advanced tools for crop breeding data integration, bioinformatics and statistical analysis. Praiseworthy work is being done towards this end by the CGIAR centres. The Bill and Melinda Gates Foundation (BMGF) has generously contributed to the Genomic Open-source Breeding Informatics Initiative (GOBii) (23) and IBP (Integrated Breeding Platform (IBP) (24), which are being run in collaboration by a consortium of national and international public sector and private sector organizations. The success of biotech-incubators within public-sector centres of excellence in disseminating modern technologies to the SME seed industry would be obvious with a glance over the list of past and present beneficiaries of the agribusiness-incubator at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (25).

Cheaper sequencers from Illumina and Ion Torrent (Fisher Scientific) are already within the purchasing power of SME seed companies (8). MinION from Oxford Nanopore Technologies, at \$1,000, could be an ultimate democratization of the sequencing technologies. Even with high error rate, the MinION data has been used successfully for diagnostic DNA fingerprinting of organisms with small genomes (26,27) and has shown promise for DNA fingerprinting of the large human genome (28). Improvements in nanopore chemistry, base-calling algorithms and library preparation protocols are expected to further improve the nanopore sequencing (29). Substantial savings can be made on infrastructure by using cheap alternatives such as paint shaker in place of sample grinding machine, black cardboard enclosure+transilluminator+digital camera as gel photo-documentation system and using -20C freezer in place of -80C freezer where feasible. It is possible to build a laboratory for DNA extraction and genotyping capable of low to medium marker coverage, without a sequencer, within \$100,000 and for low through medium to ultra-high marker coverage within \$150,000, inclusive of a sequencer (personal figures), which should be within the spending power of SME seed industry (8).

While large seed companies are playing a priceless role in feeding an ever-growing population and contributing to the development of innovative technologies for crop improvement, the success of SME seed industry in the developing countries is inextricably linked to global food security. The future of seed industry lies in tapping on large germplasm base for breaking heterosis barriers, breeding for quality traits, incorporating biotic and abiotic stress resistances and developing crops adapted to a changing climate. Modern plant biotechnological approaches would be required by the SME seed industry to perform its role well and to remain competitive. Democratizing access to modern technologies and resources for the SME seed industry in the developing countries deserves world focus.

REFERENCES

1. Padulosi S, Hodgkin T, Williams JT & Haq N. Underutilized Crops: Trends, Challenges and Opportunities in the 21st Century. *Managing Plant Genetic Diversity* 2014:323-338.
2. Sehgal S. Agricultural Biotechnology and the Seed Industry: Some Implications for Food Production and Security. In: Qaim M, Krattiger AF & Braun J, eds. *Agricultural Biotechnology in Developing Countries: Towards Optimizing the Benefits for the Poor*. Boston MA: Kluwer Academic Publishers, 2000:223-228.
3. Bijman WJJ. How biotechnology is changing the structure of the seed industry. *Agricultural Economics Research* 2001;3:82-94.
4. Gruber K. Agrobiodiversity: The living library. *Nature* 2017;544:S8-S10.
5. Xu Y, Li P, Zou C, et al. Enhancing genetic gain in the era of molecular breeding. *Journal of Experimental Botany* 2017;68(11):2641-2666.
6. McDougall P. R&D trends in Crop Protection. ABIM Conference 2012;1-15. http://www.abim.ch/fileadmin/abim/documents/presentations2012/ABIM_2012_6_McDougall_John.pdf
7. Munyikwa T. Challenges of crop production, impact of emerging technologies and role of regulation. Syngenta 2016. <http://nas-sites.org/biotech/files/2016/07/Tichafa-Munyikwa-Presentation.pdf>
8. Pray CE & Nagarajan L. Role of biotechnology in stimulating agribusiness R&D investment in India. *AgBioForum* 2013;16(2):104-111.
9. Semagn K, Babu R, Hearne S, & Olsen M. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Molecular Breeding* 2014;33(1): 1-14.
10. Rasheed A, Hao Y, Xia X, et al. Crop Breeding Chips and Genotyping Platforms: Progress, Challenges, and Perspectives. *Molecular Plant* 2017;10(8):1047-1064.
11. Bajgain P, Rouse MN & Anderson JA. Comparing genotyping-by-sequencing and single nucleotide polymorphism chip genotyping for quantitative trait loci mapping in wheat. *Crop Science* 2016;56(1):232-248.
12. Liu H, Bayer M, Druka A, et al. An evaluation of genotyping by sequencing (GBS) to map the *Breviaristatum-e* (ari-e) locus in cultivated barley. *BMC Genomics* 2014;15(1):104.
13. Yang S, Fresnedo-Ramirez J, Wang M, et al. A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: a case study for marker-assisted selection in grapevine. *Hortic Res* 2016;3:16002.
14. Buckler ES, Ilut DC, Wang X, Kretschmar T, Gore MA & Mitchell SE. rAmpSeq: Using repetitive sequences for robust genotyping. 2016. <https://www.biorxiv.org/content/early/2016/12/24/096628>
15. Vartia S, Villanueva-Cañas JL, Finarelli J, et al. A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. *Royal Society Open Science* 2016;3(1): 150565.
16. Li L, Fang Z, Zhou J, et al. An accurate and efficient method for large-scale SSR genotyping and applications. *Nucleic Acids Research* 2017;45(10):e88.
17. <https://www.lgcgroup.com/services/genotyping/#.WeF4SmiCzIU>
18. Chaikam V. In vivo maternal haploid induction in maize. In: Prasanna BM, Chaikam V & Mahaku G, eds. *Doubled Haploid Technology in Maize Breeding: Theory and Practice*. Mexico: CIMMYT, 2012:9-14.
19. Prasanna BM. Doubled haploid (DH) technology in maize breeding: an overview. In: Prasanna BM, Chaikam V & Mahaku G, eds. *Doubled Haploid Technology in Maize Breeding: Theory and Practice*. Mexico: CIMMYT, 2012:9-14.
20. Babu R, Nair SK, Vivek BS, Vicente FS & Prasanna BM. Integrating Marker assisted selection in DH-based Breeding Pipeline for Rapid Development and Delivery of Superior Parental Lines and Cultivars. In: Prasanna BM, Chaikam V & Mahaku G, eds. *Doubled Haploid Technology in Maize Breeding: Theory and Practice*. Mexico: CIMMYT, 2012:9-14.
21. Lara-Victoriano F, Castillo-Reyes F, Flores-Gallegos C, Aguilar CN & Rodriguez-Herrera R. Metagenomics in plant pathology. *Phytopathology in the Omics Era* 2011;661(2): 1-9.
22. Abdelfattah A, Wisniewski M, Nicosia MGLD, MG, Cacciola SO & Schena L. Metagenomic analysis of fungal diversity on strawberry plants and the effect of management practices on the fungal community structure of aerial organs. *PLoS ONE* 2016;11(8):1-17.
23. <http://www.gobiiproject.org/>
24. <http://www.integratedbreeding.net/>
25. <http://www.aipicrisat.org/agri-business-incubation-abi-program/about/>

26. Greninger AL, Naccache SN, Federman S, et al. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. *Genome Medicine* 2015;7(1): 99.
 27. Quick J, Grubaugh ND, Pullan ST, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nature Protocols* 2017;12(6):1261-1276.
 28. Zaaijer S, Gordon A, Piccone R, Speyer D, & Erlich Y. Democratizing DNA Fingerprinting. 2016 <https://www.biorxiv.org/content/early/2016/06/30/061556>
 29. Benítez-Páez A & Sanz Y. Multi-locus and long amplicon sequencing approach to study microbial diversity at species level using the MinION™ portable nanopore sequencer. *GigaScience* 2017;6(7):1-12.
-
-