COTTON BREEDING: DEVELOPMENTS AND OPPORTUNITIES

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ABSTRACT

It is imperative that the international scientific community understands that plant breeding is primarily an organismal science and serves as the backbone of research developments. Breeding is comprised of variety development, variety maintenance (including variety approval and certification) and seed production that have continuously changed. The private sector is increasingly involved in some of the important components of the breeding chain, and the role of the public sector has certainly diminished. A mix of different approaches is needed, and there must be agreement as to who is responsible for what. While scientific centers and seed breeding systems will be changing their modalities, they cannot ignore the fast approaching molecular breeding technologies. Marker assisted breeding and empowerment over directed breeding is the new norm of cotton breeding. Conventional breeding will be replaced with molecular breeding, a joint venture of breeders and molecular biologists.

Plant breeding started about 10,000-12,000 years ago when man observed that if a seed falls on the ground it germinates and produces a new plant (Roupakias, 2014). It is no more than 100 years ago that researchers were still struggling to accept the Mendelian Law of Inheritance and the Law of Independent Assortment. Mendelian genetics was ignored for almost 25 years due to the hesitation to admit the existence of genes or to accept that heritable characters are genetically controlled and cannot just be transferred as being acquired. The genes assort independently without any outside influence.

The extensive research done on cotton became more formal and was easier to understand after it was discovered that there are genes that carry a blueprint of the characters to be expressed under a given set of growing conditions. Such discoveries, unimaginable in the early years of cotton research, were severely questioned and remained shelved for about half a century. The theory of evolution did not satisfactorily address many concerns, and it was practically impossible to give up the long-held belief in the inheritance of acquired characters. Fortunately, however, the law of inheritance of characters and the independent assortment of genes were rediscovered and applied. Thus began the science of formal breeding we know today.

THE COTTON BREEDING OF YESTERDAY

Only three methods of breeding have been employed throughout the world, i.e. introduction, selection and hybridization. Varieties have been imported from other countries and directly adopted for commercial cultivation. This is probably the most obvious and easiest way to improve production based on improving the genetic background of cultivation material. Cotton production itself was initiated by introduction in the Indian subcontinent, it initially failed and then succeeded. The reliance on introductions has diminished since it has been understood that there is a science behind the carryover of characters and they cannot merely be manipulated based on production conditions. Introductions do not have an impressive history of success, although varieties developed in one part of the world do have a chance to excel in performance when grown under more suitable conditions within a country or across countries.

Selection from within a population, having not been produced directly through hybridization, has also proved successful in the past. The material from where selections were made often comprised adopted/commercially grown varieties. The major limitation to the selection method of developing varieties has been a lack of sufficient variability, as selection had to rely either an existing variability in the population resulting from natural out-crossing or natural mutations. Drastic deviations from existing populations were not expected and usually there was no fear of adaptation issues.

Because of the limitations described above, efforts were made to induce variability in the existing homozygous populations via mutagenesis. Chemical mutagens were employed without much success. However, radiation was extensively used in a hit and miss fashion in many countries in the 1960s and 70s. Gamma rays were more effective than other sources of radiation on cotton for desirable mutations. A number of different doses were tried, and it was found that 30 and 35 krad induced chromosomal changes with a minimum number of deleterious mutations. Pakistan probably benefitted the most from gamma radiation in the form of developing heat-tolerant varieties. The variety NIAB-78, developed through radiation was once planted on over two million hectares in Pakistan. Although the variety showed continuous segregation at low level, apart from commercial use, NIAB-78 provided a base for developing heat-tolerant varieties, which was the most important factor limiting yield until early 1980s. There are many different ways of using radiation to induce beneficial mutations, including radiating wet/soaked seed, pollen grains, using varying doses, and using different sources of radiation; all have been tried. Scientists have concluded that the high number of deleterious effects and undesirable linkages induced by radiation rendered this technique too costly and inefficient to continue.

The hybridization procedure has existed parallel to the other two approaches, but hybridization has not been employed on large scale for many reasons.

 Hybridization is a long process involving the crossing of two parents, selection from segregating populations starting from F₂ generation onward, and attaining genetically pure breeding lines before they are carried forward for commercialization.

- Populations that segregate starting from the F₂ generation have to be grown in sufficient numbers so that the maximum number of combinations can express themselves. A small F₂ population means that all the possible combinations cannot occur in the field, and subsequent generations will also be limited in the number of expressions they can exhibit, either through the bulk selection method or with progeny rows. Managing large segregating populations and various segregating generations of each population require huge resources.
- Selection from segregating populations in the field requires vigilant breeders' judgment on what should be carried forward and what not. Fiber quality was used as a criterion for rejecting single-plant selections or bulk populations, but only when the rest of the material had been discarded. It was common that breeders always had difficulty in deciding which plants and progenies to reject, and it was easier for them to select more rather than fewer plants and lines. The cost of carrying huge populations from one generation to the next restricted attempts to try more combinations and crosses.
- Nevertheless, hybridization continues as a modern method of developing varieties, but the practice relies mostly on the experience and judgment of breeders, rather than science. In other words, breeding is practiced more as an art of selecting better plants that will continue performing better in subsequent generations and will become commercial varieties.
- Unfortunately, breeders lacked a precise test that would ensure success. However, a big team of breeders attempting a large number of crosses has a higher chance for success because they are screening more combinations. Nevertheless, there is no way to guarantee that large teams will develop a superior variety than a small group of breeders attempting a few crosses every year.
- Mutagenic control of various characters, negative correlations among desired characters, and other factors complicate and restrict the flow of varieties. There were gene combinations that every breeder desired to introduce into varieties, but they were not successful because they could not pick and choose genes carrying the desired characters.

Various efforts were made to overcome some of the constraints to successful breeding of superior lines. The efforts included using backcrossing to retain or transfer a limited number of characters, the same technique now used to transfer biotech genes. Single crosses, double crosses, varieties crossing with segregation populations and many more options have been tried, but only back crossing proved its worth. The development of varieties with single, double and even triple biotech gene transfers is possible because of the experience learned from conventional breeding.

WHAT BREEDING IS CURRENTLY GOING THROUGH?

Breeding is increasingly conducted within the private sector, although it will take many more years until this shift is complete. The public sectors in many countries see private sector breeding as a challenge to their authority and have sometimes been reluctant to cooperate.

Often times, public sector breeding programs have been judged in terms of the number of varieties released for commercial production and the area planted to such varieties. Some

breeders fear that financial support from governments will dissipate if they surrender the right to commercialize varieties. However, the shift to private sector breeding is a reality, and this shift allows public sector resources to be better utilized in other areas of research that require higher attention than received in the past. Variety approval and seed certification are two aspects of the planting seed development chain that could stay with the public sector.

Breeding programs, whether in the private sector or public, are faced with issues that will have long term consequences if proper measures are not taken. Constraints are often openly discussed, but solutions are not given, or if solutions are mentioned, pathways are not shown to resolve them. Hence the elimination of constrains is not expected soon, and the consequences are potentially severe and long lasting.

The major issues confronting cotton breeding programs are:

- The genetic base of current varieties is narrow. Only a limited circle of varieties are hybridized to produce newer varieties, hence the breeders end up with only a slight change of achieving improvements over existing varieties. Individual companies or public sector breeding teams are using their own varieties for the sake of maintaining their known and accepted stylized series. They are doing so to have a higher success rate in commercializing new varieties than they would have if they attempted crossing two diverse genotypes. So, breeders themselves have fused this problem into their breeding programs, and they are the ones who must change their approaches. A breeding process can produce change in the composition of a population only if there is variation from which to select.
- Germplasm exchange has almost disappeared. Germplasm availability is the extent
 of freedom that a breeder has to obtain and use any genetic material that exists in
 or outside a country. The result of stringent plant variety protection in each country
 is restricting breeders' rights to freely use any genetic material. These restrictions
 inhibit further development of innovations. It is true to some extent that while some
 countries have been collecting significant amounts of new germplasm, they are not
 reporting significant rates of providing germplasm beyond their own borders. No
 statistics are available to report in cotton, CGIAR data show the trend in other
 crops.
- The advent of biotechnology and intellectual property laws has increased the market for improved seed. These changes made it easier for commercial breeders/companies to be rewarded and to recover the cost of their investments, But, at the same time, intellectual property protections limit the ability of farmers or rival breeders to reproduce seed. Private seed companies have benefitted the most from the expansion of the seed industry to more than \$2 billion US dollars. Public sector breeders and programs have not been compensated for their work to the same degree as private programs, resulting in shrinkage of public sector breeding programs.
- The focus in cotton breeding has shifted to short-term objectives to achieve quick returns. Budgets for long-term fundamental scientific research are shrinking, which is not in the ultimate best interest of breakthrough achievements.

The rise of molecular genetics, particularly with the commercialization of biotech crops, has accelerated the shift toward private sector plant breeding. Many scientists believe that as conventional breeding is replaced by biotechnological approaches, public sector programs and institutions will retreat from classical plant breeding. This, in turn, has a negative effect on breeding education at universities and the research work at public institutions (Roupakias, 2014). Biotechnology is relatively new and can be done in large centralized laboratories; there has been a rapid expansion of biotechnology research. That research is essential, but there has also been a reduction in public sector plant breeding efforts, which could result in a lack of progress in the development of elite germplasm and effective commercial cotton cultivars (Constable, 2015). Conventional breeding and molecular genetics are complementary and both are needed to fundamentally improve cotton varieties.

COTTON BREEDING OF TOMORROW

Cotton breeding is in a high-transition stage in which the way that genetic principles are applied is changing. It is quite possible that 'conventional breeding' together with transgenic breeding, complimented with even newer developments in biotech approaches, will find a common name. The name for the new breeding approach is not known, but it might be something like 'directed breeding,' wherein the breeder will have a specific, predetermined target, and the breeder will hybridize with certainty. Future breeders will not be working with hit and miss trial methods and with an unlimited wish list of targets. Rather, breeders will have a source for the anticipated outcome.

The long process of selection, currently followed for the sake of producing a homozygous population, has to be shortened. The production of haploid plants, and the doubling of chromosomes in the cotton genome, have long been targeted, but additional options may become available with the progress we are making. The other time consuming aspect of breeding is multi-location performance testing of varieties under varied sets of agronomic practices. This also needs to be changed. It is not economical to test candidate varieties at many locations, under various sets of agronomic situations, and then make selections based just on normal conditions.

Cotton, especially *G. barbadense*, is highly sensitive to photoperiodic complexities and thermal conditions. Researchers have talked about 'global varieties' that will perform equally well anywhere in cotton producing areas. Imagine if a good team of breeders is allowed to focus on innovative developments rather than breeding varieties based on luck and having to wait 12-14 years to know the fate of their breeding lines.

As a breeder myself years ago, I recall that plenty of exceptional germplasm lines were deficient in only one or two traits, but those traits were so important that the germplasm lines were discarded. Discarding such genotypes, which is routine, is a waste of genetic resources. Useful gene(s), for example genes for fiber length or strength, which are deficient in an otherwise exceptional germplasm line can be transferred through genetic engineering, for which molecular markers are available or could become available. The deficient lines can be used as recurrent parents for marker assisted accelerated back-cross breeding methods. Transgenic breeding has already allowed researchers to find suitable genes in related and non-related species and induct them into their desired lines.

The biggest change is, of course, going to come from biotechnology applications. It is obvious that many more biotech cotton varieties with single-gene and pyramid-gene traits will be available soon. These varieties will not be limited to the herbicide tolerant or insect resistant traits currently available, but will exhibit unique features. Over the next 10, 20 or 30 years, breeding will be employed to transfer specific new traits into current varieties. Quoting many researchers, Abdurakhmonov (2013), stated that the 21st century's "omics" science and innovative genomics tools are the most promising approaches, in combination with contemporary cotton breeding knowledge and strategies. The strategies include (1) accelerated development and success of transgenic, cisgenic and intragenic biotech crop technologies with complex effects targeted to improve the intrinsic yield in cotton, and (2) decoding of cotton genomes and the mapping and characterization of the genetic basis of complex traits (referred to as quantitative trait loci-QTLs) that provide better exploitation of existing genetic diversity of cotton germplasm and gene pools, and a widening of the genetic diversity of commercialized cotton cultivars using modern marker-assisted selection (MAS), marker-assisted backcross selection (MABS) and genomic selection (GS) programs.

Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers (Jiang, 2013)). Classical markers include morphological markers, cytological markers and biochemical/protein markers. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005), such as RFLP (Restricted Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), SNP (Single Nucleotide Polymorphism), etc.

The use of marker assisted technology will be most easily employed when a particular feature is controlled by a small number of genes, and their impacts are influenced little by ambient conditions. Unfortunately, many genes control lint yield and fiber quality properties, each inducing only a small effect. For example, Shen *et al.* (2011) stated that advanced-backcross quantitative trait locus (QTL) analysis of an interspecific *G. hirsutum* × *G. barbadense* population, showed that 28 fiber length QTLs were identified including qFL-chr1 on chromosome 1 of the A-sub genome. The *G. barbadense* allele at this QTL contributed to longer fibers and explained up to 24% of the phenotypic variance. Managing these quantitative traits is even more difficult because of the number of genes involved. DNA markers associated with QTLs for improved fiber quality such as length, strength and uniformity will be explored for Pima cotton. It might take many years to overcome challenges, such as the simultaneous improvement of yield and fiber quality, but molecular technologies will certainly accelerate the process of improving the cotton genome. The Cotton Marker Database at http://www.cottonmarker.org/cgi-bin/cmd search marker result.cgi, has hit 9,027 records, and most of them were reported

after 2000 and many in the F_2 or BC₁ populations.

Malik *et al.* (2014) have presented a good review of the role of molecular markers in cotton genetic improvement, including future prospects for the practical utilization of new molecular technologies. An overview (of literature) of genetic diversity studies in cotton

using different kinds of markers, i.e. RAPDs, SSRs, AFLPs, ISSRs (Inter Simple Sequence Repeats) and SNP, showed that thousands of upland and barbadense populations, and a number of diploid cultivated and uncultivated species genotypes, have been studied in the USA, China, India, Pakistan and many other smaller cotton producing countries. Enormous work is going on to develop more efficient DNA markers for plant breeders and geneticists for developing cultivars of cotton in more efficient ways. It is hoped that SNPs markers will have large influence on molecular assisted selection and mapping studies in the future due to an abundance of sophisticated detection systems that will be developed.

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