

*African Journal of Rural Development*, Vol. 3 (3): July-September, 2018: pp.849-858. ISSN 2415-2838

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## Progress and future prospects in groundnut improvement to feed Africa in the face of technological advancements

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### ABSTRACT

Crop productivity is crucial in meeting food demands to feed the growing population in the face of endemic biotic and abiotic stresses. Technological advancement and its application to boost crop productivity would be a pathway towards ensuring food and nutrition security. Dryland legumes including groundnut are suitable in diversification of farming systems as insurance crops to ensure productivity. Crop improvement is one of the pillars towards enhancing productivity by delivering products and services based on demand articulation such as high yielding resilient varieties that are nutrient dense to address the global nutrition agenda. Recent advancements in molecular technology has made it possible to sequence the groundnut genome, develop genetic maps and identification of quantitative trait loci (QTLs) for key traits of importance. These new developments need to be exploited to accelerate the design and development of quality products that fits within the African farming systems. The low genotyping cost has opened avenues for research centers in African countries to embrace the use of genomic selection tools in breeding. This should enhance efficiency in exploiting the wild genetic resource base, broadening the narrow genetic base of groundnut and fast tracking variety release. The use of molecular tools in breeding and wide hybridization techniques coupled with high throughput phenotyping is a new dawn to breeding programs and this would contribute significantly to food security and poverty alleviation in the long run. However, the success in the modernization of breeding for efficiency will be underpinned by pro-active engagement among different actors in the national, regional and international arena to leverage resources and expertise in the omics era for sustained outcomes. Healthy working partnerships are also key to the delivery and utilization of such technologies coupled with learning and feedback for product improvement.

Key words: *Arachis hypogaea*, breeding efficiency, genomic, genotyping, product development, productivity, sequencing

### RÉSUMÉ

La productivité des cultures est cruciale pour répondre à la demande alimentaire afin de nourrir la population croissante face aux stress endémiques biotiques et abiotiques. Les progrès technologiques et leur application pour accroître la productivité des cultures seraient un moyen de garantir la sécurité alimentaire et nutritionnelle. Les légumineuses des zones arides, y compris l'arachide, conviennent à la diversification des systèmes agricoles en tant

**Cite as:** Mwololo, J., Odong, T. and Okori, P. 2018. Progress and future prospects in groundnut improvement to feed Africa in the face of technological advancements. *African Journal of Rural Development* 3 (3): 849-858.

Received: 21 April 2018

Accepted: 14 August 2018

Published: 30 September 2018

que cultures d'assurance pour assurer la productivité. L'amélioration des cultures est l'un des piliers de l'amélioration de la productivité grâce à la fourniture de produits et de services fondés sur une articulation de la demande, tels que des variétés résilientes à rendement élevé, riches en nutriments pour répondre aux préoccupations mondiales en matière de nutrition. Les progrès récents de la technologie moléculaire ont permis de séquencer le génome de l'arachide, d'établir des cartes génétiques et d'identifier les locus de traits quantitatifs (LTQ) pour les principaux caractères importants. Ces nouveaux développements doivent être exploités pour accélérer la conception et le développement de produits de qualité qui s'intègrent dans les systèmes agricoles africains. Le faible coût du génotypage a ouvert aux centres de recherche des pays africains les moyens d'utiliser des outils de sélection génomique pour la sélection. Cela devrait accroître l'efficacité de l'exploitation de la base de ressources génétiques sauvages, en élargissant la base génétique étroite de l'arachide et en libérant rapidement les variétés. L'utilisation d'outils moléculaires dans les techniques de sélection et d'hybridation à grande échelle, associée au phénotypage à haut débit, constitue une nouvelle avancée pour les programmes de sélection, ce qui contribuerait de manière significative à la sécurité alimentaire et à la réduction de la pauvreté à long terme. Cependant, le succès de la modernisation de la sélection pour l'efficacité sera soutenu par un engagement actif des différents acteurs aux niveaux national, régional et international afin de mobiliser les ressources et l'expertise à l'ère de l'omique pour obtenir des résultats durables. Des partenariats de travail sains sont également essentiels pour la fourniture et l'utilisation de ces technologies, ainsi que pour l'apprentissage et le retour d'informations afin d'améliorer le produit.

Mots clés: *Arachis hypogaea*, efficacité de la sélection, génomique, génotypage, développement de produits, productivité, séquençage

## **INTRODUCTION**

In sub-Saharan Africa, climate variability has become a dominant phenomena in the recent years with an irregular distribution of rain and temperature regimes. Agricultural productivity in the semi-arid tropics characterized by marginalized populations, is threatened by global challenges ranging from weak environmental resource base, population increase institutional and technical capacity, among others. Enhancing land and crop productivity to supply food to improve general well-being of low-income people is, therefore a priority. Numerous studies have elucidated the link between agriculture, food, and nutrition security and a number of conceptual frameworks developed as a road map on how agriculture can be exploited to contribute to the nutrition agenda (Dorward, 2013). Productivity can be unlocked through technology development and scaling,

modernization of agricultural research owing to advancements in molecular biology, promotion of environmentally sustainable agronomic practices, and refining research focus to include both input and output traits such as nutrient content in final research products. An entry point to underpin production and farmer livelihood strategies would be deployment of high yielding resilient legume crops, supported by modern breeding approaches to accelerate the design and development of such superior products. Genetic intensification in dryland legumes to deploy superior varieties in production systems in Sub Saharan Africa, is a pathway to enhancing food and nutrition security while availing climate variability adaptation measures at the farm level. Dryland legumes once incorporated in cereal cropping system lead to multiple benefits as resilient crops that provide dietary diversity for local consumption, act as cash crop, or as feed

and fodder for livestock. The major legumes grown in the East and Southern Africa region includes chickpea, mungbean, common bean, groundnut, pigeonpea, soyabean and cowpea. Legume improvement for key output traits of preference such as nutrient content to meet nutrition needs for households in the region can be achieved through breeding. Genetic enhancement for legumes is possible due to the availability of diverse germplasm sources in gene banks across the world from where to tap superior alleles.

**Groundnut.** Groundnut (*Arachis hypogaea* L.) belong to the family Fabaceae and is an annual crop distinguished from most other legume crop species by producing aerial flowers and fruiting below ground. Botanically, groundnut falls into two subspecies namely, subsp. *fastigiata* and *hypogaea* (Krapovickas and Gregory, 1994). The crop is made up of “AA” and “BB” genomes arising from *Arachis duranensis* and *Arachis ipaensis*, respectively (Moretzsohn *et al.*, 2012).

Groundnut is grown for oil, food, feed and as a soil fertility nourishing crop across the world. Africa and Asia are the leading producers of the crop. Production statistics as of 2015 indicated that 24 million ha area worldwide was under groundnut whereby 42.4 million tons were produced (FAO, 2016). The crop is cultivated under rain fed conditions with low input use, a characteristic common among smallholder farmers in the Semi-Arid areas. Groundnut provides food and dietary needs in most households.

**Groundnut production constraints.** Groundnut production at farmers’ fields is constrained by a variety of factors. Similar to most other crops, a big yield gap exist between genetic potential of improved groundnut varieties and their actual yield in the field; with yields varying between 500-700 kg/ha compared to yields of over 4

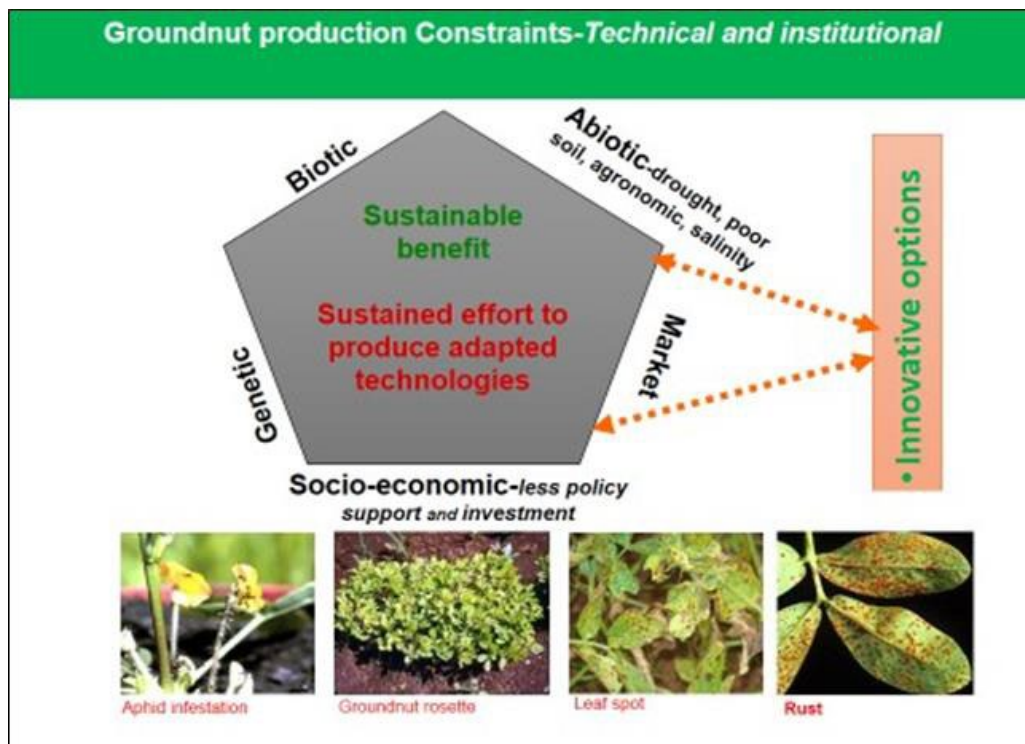
tons/ha obtained on research stations. Among the major constraints are biotic and abiotic stresses. The key biotic stresses include foliar diseases which have fungal or viral origins. Yield losses are generally substantial when the crop is attacked by diseases (Nigam *et al.*, 2012). The early leaf spot disease caused by *Cercospora arachidicola* Hori, late leaf spot (*Cercosporidium personatum* Berk. & Curtis.) and rust disease caused by *Puccinia arachidis* Spegazzini are the most prevalent fungal diseases of the crop (Liu *et al.*, 2013). Leaf spots cause yield losses ranging between 50–70% in susceptible varieties (Khedikar *et al.*, 2010). The major devastating viral disease is the groundnut rosette disease caused by a virus complex that includes two viruses and a virus-like nucleic acid molecule known as the satellite RNA. Different forms of satellite RNA exist that lead into three types of symptoms in the field namely: chlorotic (yellowing), mosaic and green rosette. The most important pre- and post-harvest insect pests of economic importance in groundnut include Aphids (*aphidoidea*), thrips (Tysanoptera), Jassid (*Amrasca devastans*) and Spodoptera (*Spodoptera litura*). Weeds as a pest cause considerable yield reductions when not controlled early in the season (Murata, 2001). They key abiotic constraints include water stress, heat stress and soil fertility that are prerequisite for crop growth and development. Lack of improved varieties that are adapted to these stresses is an additional challenge that face farmers. A summary of the production constraints is presented in Figure 1.

**Addressing groundnut production constraints.** One way of increasing productivity and improving sustainability is through the use of improved cultivars. Consequently, efforts have been put on yield improvement through genetic enhancement for resistance and or tolerance stresses. Breeding speed is reduced by the nature of complex traits to deal with and linkage drag. There are inadequate high

throughput phenotyping methods and in some cases farmers are reluctant to use improved technologies possibly due to poor product design and delivery models. Across the world, groundnut improvement programmes have been engaging in research to develop varieties that meet the preferences of different users along the value chain. Owing to malnutrition, nutrient dense groundnut varieties would contribute towards the nutrition agenda. Substantial efforts towards breeding for these traits are ongoing. The nutrient components of importance are iron, zinc, oil content and oleic acid. Progress in breeding through selection ride on genetic variability and groundnut has a challenge due to inherent low genetic variability, and limited cross-compatibility with wild diploid species. Despite the highlighted constraints and associated challenges, opportunities exist such as modern breeding approaches to tag key genes of interest and their introgression through

marker assisted selection to speed up delivery of superior varieties; availability of genetic information such as sequenced maps and refined genomes, existence of genetic diversity including exploitation of wild relatives and the upsurge of new innovations such information technology based breeding management systems.

**Progress in the use of modern breeding tools in groundnut improvement.** Appreciable success in boosting groundnut productivity have been attained through conventional breeding, though at a slow pace. Consequently, building on the existing success, crop productivity can be enhanced further through intensification of genetics using molecular tools. To that end, genomics can be exploited to accelerate the development of improved cultivars. In this era of gene technology, the refinement genetic resources for use in groundnut improvement



**Figure1. Summary of key production constraints in groundnut production in Africa**

programs is taking shape. For instance, technologies such as next generation sequencing (NGS) and genotyping have been invented for use in associating markers to traits, studies to discover genes coupled with their functions to develop function specific markers that would accelerate the breeding process. Currently, several experts have documented the success and use of these genomic resources in improvement of legumes (Bohra *et al.*, 2014; Varshney, 2016). Most of the progress in the generation of genetic information of groundnut has now been made possible in recent advancements whereby new molecular markers have been developed, genetic and physical maps that are dense generated, refined expressed sequence tags (EST) identified, important genes discovered and chromosomal regions (QTL) linked to stresses of economic importance identified (Manish *et al.*, 2016). This has further led to the application of molecular markers in breeding (MAB) to complement conventional methods, culminating into the release of superior groundnut varieties. Following the adaptation of MAB, multiple trait lines have been developed (Varshney, 2016). Additional resources that have strengthened the field includes a marker-trait association platform as an avenue for integration of genomic tools in groundnut breeding for quick genetic gain and delivery of quality finished products in form of superior varieties. For instance, genomic selection (GS) have been employed in improvement for resilient to drought and yield that are complex traits governed by a number of minor effect QTLs. Recent advancements have also led to the generation of the groundnut draft genome sequence and new cheaper genotyping tools (e.g. genotyping by sequencing). Such advancements if adapted would trigger the use of modern genomic tools to intensify genetics for traits of economic importance. The use of these tools in Sub-saharan Africa is at its nascent stage, hence the need to exploit this opportunity to accelerate breeding for efficiency.

**Genomic sequencing in legumes.** Current research advancements in genomics offer an array of molecular based tools for integration in breeding programs to revolutionize crop genetic intensification to deliver superior products that would enhance production. The inventions of new approaches such as the next generation sequencing (NGS) technology based approach offer the benefit of reduced time and cheaper genome sequencing compared to the earlier methods (Schatz *et al.*, 2002). The use of the NGS technologies hastened the genome sequencing at a lower cost and this has made it possible to decode complex genome sequences. So far more than one hundred and fifty (150) genomes have been sequenced (Michael and Jackson, 2013). These recent strides made in decoding the plant genome sequence was a long awaited opportunity to dissect and elucidate functional characterization of genes at the molecular level.

There was little focus on genomics resources for dryland legumes until recently when these crops have been recognized for their importance in farming systems (Varshney *et al.*, 2012). As a result, the International Peanut Genome Initiative (IPGI) undertook to sequence groundnut resulting into sequenced genomes of the diploid groundnut progenitors. These progenitors included *Arachis duranensis* and *Arachis ipaensis* representing the A and B genomes respectively (Bertioli *et al.*, 2016). This is expected to facilitate exploitation of molecular approaches in groundnut improvement. Integration of phenotypic and genomic studies would help in shedding more scientific light about the genome architecture and the physiological/biochemical mechanisms associated with response to stress. Genetic diversity would further support this advancement, given the large variable untapped germplasm resources available in gene banks, thus room to mine superior alleles for genetic intensification in the cultivated gene pool, product development

and delivery (McCouch *et al.*, 2013). Through such efforts, the enhanced advancements will help in refining the identification of superior sources of traits of economic importance. For instance, the international crops research institute (ICRISAT) and other countries such as USA and UK are endowed with thousands of dryland legumes germplasm from hundreds of countries (Gowda *et al.*, 2013). Combination of genomic research with other approaches such as biotechnological tools to develop transgenics and the use of synthetic allotetraploids would help in increasing the genetic diversity available for exploitation in breeding for quick genetic gains.

#### **Genetic mapping approaches towards adaptation of molecular tools in groundnut breeding**

**Quantitative trait loci mapping.** The analysis of quantitative trait locus (QTL) aim at linking genotypic and phenotypic data sets in an effort to give more information on the genetic basis of variation in complex traits (Falconer & Mackay, 1996). Genetic mapping enables researchers in life sciences to link complex phenotypes to specific chromosomal regions. This is important in tagging of the genomic regions that are linked to key traits of interest before extensive use of the linked markers in marker-assisted breeding. The use of molecular markers has far reaching benefits if integrated in groundnut breeding programmes since leads to quick genetic gains due to ability to select for quantitative traits, reduction of linkage drag while transferring superior traits from wild relatives and generation of chromosome segment substitution lines through genome wide introgression which are crucial in broadening the narrow genetic base of the groundnut crop. In addition, it contributes to rational utilization of resources since phenotyping is minimized and only done at strategic stages and there are no chances of phenotype screens and escape situations. At

the same time, using molecular markers as tracking tools, it is possible to design a breeding programme that targets multiple traits such as yield, disease resistance, and quality traits among others in groundnut breeding.

**Marker assisted back crossing as a molecular breeding approach.** Much success has been achieved in gene pyramiding using marker assisted backcrossing in the development of improved lines and or varieties. The very first work on the use of marker assisted backcrossing in groundnut breeding focused more on resistance to biotic stresses and quality (oil) following the identification of markers and quantitative trait loci with high phenotypic effect (Varshney, 2013). In the present, marker assisted breeding has widely been used to generate near-isogenic lines and chromosome substituted lines (CSSLs) for further use in genomic research advancements. The limiting factor is that, a large population is required in marker assisted backcrossing and therefore it is impossible to handle multiple traits/QTLs at one go (Varshney *et al.*, 2012). The marker-assisted recurrent selection (MARS) is the most suitable molecular approach for handling drought and yield traits that are regarded as complex (Ravi *et al.*, 2011; Gautami *et al.*, 2012; Ribaut and Ragor, 2017). However, the major drawback to date has been the absence of dense linkage maps and this limits the applicability of this approach in breeding (Ravi *et al.*, 2011).

**Multi-parent mapping population in molecular breeding.** The creation and use of multi-parent mapping populations is a major shift to facilitate handling of more traits in one population. The two major types of populations which has made it possible to proceed in this direction includes nested association mapping and multi-parent advanced generation inter-cross (MAGIC) populations. In the MAGIC, a set of diverse parents with varying traits of interest are required to generate these populations

(Varshney and Dubey, 2009); and such give more polymorphic information content when screened with markers and rich genetic maps with high incidence of quantitative trait loci, and the constituting of multiple traits within a single population (Manish *et al.*, 2016). Using the advanced intercross line population method, diverse lines are selected and intercrossed randomly (Darvasi and Soller, 1995); and this lead to breaking of linkage between very closely linked loci due to the occurrence of multiple recombination events. Although these mapping populations have largely been used in fine mapping for a number of crops; their use in groundnut breeding is just taking off (Huang *et al.*, 2010). These populations provide more precision in the detection and estimation of chromosomal regions associated with key traits and are therefore useful in groundnut breeding modernization.

The nested association mapping exploits and builds on the benefits associated with linkage and association mapping, where it is able to tag and dissect exact markers for key complex traits such as yield and drought. However, the level of success is dictated by the frequency of the functional marker alleles and their level of contribution to the genetic effects, the disequilibrium share between the functional and non-functional markers, the methods adapted for statistical analysis, and the choice of mating design (Guo *et al.*, 2010). Under this kind of population, diverse recombination events are integrated to exploit the benefits of low marker density, high density of useful alleles and high level of mapping resolution and the power of statistics generated. The approach includes several parental genotypes that contribute to the generation of the population and this provide high chance of reconstituting more functional markers as opposed to bi-parental populations.

Multiple parents ranging from four to eight of diverse origins possessing desirable traits such

disease resistance, drought tolerant, high yield and quality characteristics can be intercrossed with the aim of constituting these multiple favorable alleles into one population in the long run. This approach is known as a multi-parent advanced generation intercross (MAGIC) population and it is currently in use in groundnut breeding programs. It forms a basis for trait based association mapping and at the same time contributes to broadening the genetic base in groundnut breeding, a key requirement in breeding for quick genetic gains. Further, such a population would form a basis for ne mapping and in dissection of the complex architecture of traits (Cavanagh *et al.*, 2008). The concept of MAGIC population is therefore an important resource in groundnut breeding in the discovery of superior genes, their isolation and transfer, an exercise which integrates both conventional and molecular breeding.

**Sequence based trait mapping.** Following the advancements in modern genomics, trait mapping and getting a subset of plants in a population at the molecular level is possible, owing to the next generation sequence (NGS) technologies coupled with reduced sequencing cost. Additional advantage to this NGS technologies is that it is timely and therefore possible to get data within the shortest time possible compared to earlier traditional trait mapping approaches. Such development has led to adaptation of this approach in genetic mapping and several sequencing-based approaches have been developed for use in genetic mapping of key traits. The use of genomic selection is a dawn of new era in crop improvement programs since it is a more advanced approach that select lines based on the entire genomes as opposed to marker assisted back crossing and marker assisted recurrent selections that focus on few genome segments. The genomic based sequencing (GBS) has the benefit of identifying large number of genome wide SNPs within a short period for use in genetic diversity studies,

genetic mapping, genome wide association mapping and genomic selection in crops, including groundnuts (Elshire *et al.*, 201; He *et al.*, 2014). The adaptation of this approach in crop improvement programs is due to the fact that the genotyping cost to generate high-density molecular data is affordable. This has seen the application of the GBS approach in a number of marker based studies such as analysis of genetic diversity (Fu *et al.*, 2014), construction of genetic maps (Kujur *et al.*, 2015; Li *et al.*, 2015), targeted genomic region selection (Jaganathan *et al.*, 2015), marker-trait linkage studies (Romay *et al.*, 2013) and in molecular based selections (Poland *et al.*, 2012, Crossa *et al.*, 2013; Huang *et al.*, 2014).

**Progress in use of molecular markers in groundnut breeding.** Within the ICRISAT global breeding programme, efforts have been made to deliver groundnut varieties that are high yielding coupled with resistance to early leaf spot, groundnut rosette diseases, rust and late leaf spot. Major quantitative trait loci (QTL) for rust resistance (QTLrust01) were identified in earlier efforts to integrate molecular markers in the global breeding program. Using marker assisted backcrossing approach these QTLs were introgressed into popular groundnut varieties to introduce rust resistance. A number of selected superior lines are being advanced into finished product and are now under evaluation for adaptability and QTL stability across Africa. Further work using MABC, included the incorporation of mutant fatty acid dehydrogenase (FAD) genes to the A and B genomes that control high oleic acid (Janila *et al.*, 2012). In addition, marker assisted gene pyramiding has been initiated following the identification of major QTL for rust and late leaf spot (LLS) resistance which explained a substantial phenotypic variation (Sujay *et al.*, 2012). More work on identifying QTLs linked to groundnut rosette disease (GRD) are under way following a breakthrough in identification

of sources of resistance and development and release of GRD resistant lines.

Wide hybridization techniques can be exploited to overcome the linkage drag barrier associated with wild species in groundnut breeding. The genome wide introgression and advanced backcross mapping are molecular marker-based approaches that would offer a solution to exploit the alleles from wild species for use in crop improvement particularly in groundnut that has a narrow genetic base. This approach has been successful in introgression of favorable QTLs in tomato and rice (Wang *et al.*, 2005; Liu *et al.*, 2006; Schmalenbach *et al.*, 2009), thus a clear indication that it can be adapted in groundnut breeding. In groundnut, the attempt to use AB-QTL approach was done by Foncéka *et al.* (2009) who developed a genetic linkage map of wild genome for introgression into the cultivated background, and at the same time generated CSSLs and AB populations. This forms a basis for advancing the work further by adopting such products into groundnut breeding; for instance by screening different segments of the wild species genome contributing to drought tolerant and quality traits. Once these segments are tagged and linked to key traits of importance, molecular markers for the same would be refined for use in groundnut breeding programs. Similar efforts to develop AB-QTL populations are underway at the center of genomics the International Crops Research Institute for the Semi-Arids Tropics (ICRISAT).

## CONCLUSIONS

Over the years, a couple of improved high yielding resilient groundnut varieties developed through conventional breeding have been released for cultivation globally. Following advancements in modern technology, molecular marker linkage maps for groundnut have been developed. Such a breakthrough has paved way for the integration of molecular tools in groundnut breeding. Cheaper sequencing



technologies are promising since this would complement conventional breeding efforts. Over the years, heart of science genomic tools have been developed that span through genetic mapping for desirable traits, discovery of genes linked to key biological processes, and gene introgressions targeting specific traits in crops including groundnut. The marker technologies offer approaches that would help the research community to tap under-utilized desirable alleles from wild *Arachis* species. The adaptation of molecular based approaches is gaining momentum in the developing countries due to infrastructure improvement, cheaper genotyping services and existence of substantial human capacity in genotyping.

**Future strategies for improving groundnut productivity.** There is need for adoption of sound scientific methodologies to generate variation through wide hybridization; and development of refined high density genetic maps for efficiency in marker development that would benefit breeding programs. The current and the upcoming modern technologies offer a basis for more thrust to refine the molecular breeding approaches to further support legume improvement programs to deliver superior products that have appreciable genetic gain on-farm within a short period and at an affordable cost. Refining and validation of molecular markers coupled with testing of modern breeding approaches in diverse environments for traits of importance to the farming community is a priority now and in the future to complement conventional breeding. High throughput phenotyping for precision to ensure accurate linkage of markers to phenotypic traits and the refinement of selection indices for use by breeders is of major focus and once achieved the authenticity and routine use of markers will be enhanced. Such rapid screening methods will speed up the identification of sources of resistance and or tolerant to multiple stresses and quality traits to hasten development of

resilient varieties targeting East and Southern Africa. The application of novel molecular technologies also opens streams for data, and as a result, data integration of all data-producing research activities in multi-disciplinary teams as a research modernization effort for efficiency should be done to ensure availability of high-quality data to the public in the research arena. The success in the modernization of breeding for efficiency will be underpinned by more proactive engagement among different specialists in both national, regional and international institutions to leverage resources and expertise, build capacity of African breeding program partners and exchange knowledge and lessons learned in the current omics era. Finally, enhanced product delivery and utilization should be made possible through strategic partnerships with NARS, public and private sectors to rationalize resources to support commercialization, adoption and diffusion of such technologies within the farming community.

#### ACKNOWLEDGEMENT

Support for this research was made possible through a capacity building competitive grant “Training the Next Generation of Scientists” provided by Carnegie Cooperation of New York through the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM).

#### STATEMENT OF NO-CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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