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GENOMIC AND TRANSCRIPTOMIC APPROACHES TOWARDS THE GENETIC IMPROVEMENT OF AN UNDERUTILISED CROPS: THE CASE OF BAMBARA GROUNDNUT

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ABSTRACT

With the world population estimated to be nine billion by 2050, the need to exploit plant genetic diversity in order to increase and diversify global food supply, and minimise the over-reliance for food on a few staple crops is of the utmost importance. Bambara groundnut (*Vigna subterranea* (L) Verdc.), is underutilised legume indigenous to Africa, rich in carbohydrates, with reasonable amounts of protein. It is known to be drought tolerant, able to grow on marginal lands where other major crops cannot with minimal rainfall (<700 mm) and chemical inputs. Crop improvement for abiotic stress tolerance and increasing/stabilising yield have been difficult to achieve due to the complex nature of these stresses, and the genotype x environment interaction (GxE). This review paper highlights how a number of recent technologies and approaches used for major crop research, can be translated into use in research of minor crops, using bambara groundnut as an exemplar species. Using drought tolerance as a trait of interest in this crop, we will demonstrate how limitations can affect genomic approaches for understanding traits in bambara groundnut, and, how genomic and transcriptomic methodologies developed for major crops can be applied to underutilised crops for better understanding of the genetics governing important agronomic traits. Furthermore, such approaches will allow for cross species comparison between major and minor crops, exemplified by bambara groundnut leading to improved research in such crops. This will lead to a better understanding of the role of stress-responsive genes and drought adaptation in this underutilised legume.

Key Words: Drought stress, Next Generation Sequencing, *Vigna subterranea*

RÉSUMÉ

Avec la population mondiale estimée à neuf milliards de personnes à échéance 2050, il est impérieux d'exploiter la diversité génétique des plantes afin d'accroître et diversifier la production globale en aliments, mais aussi réduire la dépendance à outrance de peu d'aliments de base pour l'alimentation humaine. Le vouandzou (*Vigna subterranea* (L) Verdc.), est une légumineuse indigène sous utilisée en Afrique, mais qui est riche en amidon, avec une quantité raisonnable de protéine. Il est reconnu comme étant résistant à la sécheresse, il est capable de pousser et de réaliser un cycle végétatif et reproducteur parfait dans les zones marginales de basse pluviométrie (<700 mm) où d'autres cultures majeures ne peuvent survivre. L'amélioration des cultures pour la tolérance face aux stress abiotiques et l'accroissement et la stabilisation des rendements ont été difficiles à réaliser en raison de la nature complexe de ces stress et l'influence de l'interaction génotype-environnement (GxE). Cette revue de littérature montre comment les nombreuses technologies et approches récentes utilisées par la recherche sur les cultures majeures peuvent adaptées et utilisées dans la recherche sur les cultures mineures, en se servant du bambara

groundnut comme espèce modèle. En prenant la tolérance à la sécheresse comme caractère désiré pour cette culture, nous allons démontrer comment les insuffisances des approches de génomique peuvent empêcher la maîtrise des caractères désirés chez le vouandzou et comment les techniques de génomique et de transcriptomique développées pour les cultures majeures peuvent être appliquées aux cultures sous utilisées afin de mieux comprendre les déterminants génétiques gouvernant les caractères agronomiques. De plus, de telles approches permettront de comparer les cultures majeures et mineures, avec ici l'exemple du vouandzou qui permettra d'améliorer le niveau de recherche chez de telles cultures. Cela permettra de mieux comprendre le rôle des gènes répondant au stress hydrique et l'adaptation à la sécheresse chez cette légumineuse sous utilisée.

Mots Clés: Stress hydrique, séquençage des générations futures, *Vigna subterranea*

INTRODUCTION

The dependence of global food security on major crops is a major concern in the future for food supply and also for rural income, as yield gains from these major crops may not be enough to sustain the estimated nine billion people on the planet by 2050 (Godfray *et al.*, 2010). Also, the danger presented by climate change, leading to increased drought, temperature, flooding, and salinisation, along with a predicted increase in pests and diseases, could drastically affect major crops growth and development. There is need to widen the exploitation of the available plant genetic diversity in order to increase food supply and avoid dependence on a limited number of plant species for global food and nutritional security. Underutilised crop could be a solution for more diversified agricultural systems, a rich source to explore novel trait values and additional food sources necessary to address food and nutritional security concerns (Jaenicke and Höschle-Zeledon, 2006; Mayes *et al.*, 2012; Williams and Haq, 2002).

With the availability of technologies, such as Next Generation Sequencing (NGS), it is possible to develop molecular markers for marker assisted selection (MAS) in underutilised crops (Moe *et al.*, 2012). This technology is able to generate significant sequence datasets, and allows in-depth comparisons to be made between underutilised crops and their major staple crop cousins (Mayes *et al.*, 2012). For underutilised crops, their low and erratic yields can be due to the lack of genetic improvement and formal breeding programmes which may be hindering their wider cultivation and utilisation (Jain and Gupta, 2013).

Several efforts have been made to conserve the germplasm of major crop species, but a more limited amount of effort has gone into conservation of underutilised crops such as bambara groundnut (*Vigna subterranea* (L) Verdc.). The Consultative Group for International Agricultural Research (CGIAR) and Global Diversity Crops Trust are organisations whose focus is to develop a sustainability plan in order to ensure germplasm conservation and availability of underutilised crops, as a means to increase crop diversity (Houry *et al.*, 2010; Anon n.d.). Conserving the genetic resources of underutilised crops exemplified by bambara groundnut, is essential as these crops are sources of livelihood in rural poor communities and its cultivation has a positive impact on farmer welfare (William *et al.*, 2016). However, conservation is only one aspect as without 'conservation into use', no impact is made on the lives of the farmers who could benefit. *Ex situ* and *in situ* germplasm conservation, with farmer participatory breeding and, identification of favourable traits, offer a productive solution to conserve and utilise the genetic resources of underutilised species. This approach can then be coupled with trait analysis to identify crops which can (i) survive in extreme conditions (e.g., drought and cold), (ii) have superior nutritional content, (iii) and have the potential to achieve high market value in order to increase their utilisation and consumption, contributing to the global food basket (iv) are acceptable to farmers

This is the first review on bambara groundnut with a specific focus on the genomic/transcriptomic approaches available to address drought through genetic improvement. This review also highlights how some recent

technologies and approaches used for major crop research, can be translated into use in research of minor crops, such as bambara groundnut. Previous reviews on genetic improvement of bambara groundnut using resources from major species has focused mainly on the overview breeding objectives and aims (Aliyu *et al.*, 2015). Other reviews published on bambara groundnut focus mainly on (i) conservation and improvement (Heller, 1997); (ii) production (Mkandawire, 2007); (iii) developing the potential of the crop (Azam-Ali *et al.*, 2001); (iv) aspects of the commodity marketing of bambara groundnut (Hillocks *et al.*, 2012); (v) breeding approaches towards the genetic improvement of bambara groundnut and (vi) genetic diversity analysis of bambara groundnut (Aliyu *et al.*, 2016, 2015).

Bambara groundnut profiles. Bambara groundnut is probably one of the most drought-tolerant of the major grain legumes and may be found surviving successfully where annual rainfall is below 500 mm and has an optimum between 900–1000 mm per year (Bamshaiye *et al.*, 2011) although it can grow in wetter conditions as long as the roots are not water-logged. It is cultivated mainly in sub-Saharan Africa, expanding to regions of West Africa, across Central Africa to East Africa and Southern Africa (Mohale *et al.*, 2013).

It is cultivated principally by farmers as a food security culture crop, because of its agronomic values and the ability to produce in soils considered insufficiently fertile for cultivation of other more favoured species such as common beans (*Phaseolus vulgaris*) and groundnuts (*Arachis hypogaea*) (Olaleye *et al.*, 2013), although there are markets and there is some early

evidence that it could improve household incomes (William *et al.*, 2016)

Bambara groundnut has reasonable protein content (18 to 22%), high carbohydrate (65%) and low levels of fats (6.5%), having a composition quite similar to chickpea (*Cicer arietinum*) and makes it a 'complete food' (Mazahib *et al.*, 2013) (Table 1). The seed commands a relatively high market price in many African countries (Coudert, 1984). In countries such as Malawi, demand for bambara groundnut often exceeds supply (Mkandawire, 2007). Bambara groundnut is considered to be the third most valuable legume in Africa, after cowpea (*Vigna unguiculata* (L.) Walp.) and groundnut (Murevanhema and Jideani, 2013). It is known to be deficient in sulphur-containing amino acids (Azam-Ali *et al.*, 2001), but rich in lysine, leucine and glutamic acid, which makes a good complement to cereal-derived amino acids (Mazahib *et al.*, 2013) (Table 2); hence in many African countries, bambara groundnut seeds are often milled and added to wheat flour and used to make a number of baked products (Abdualrahman *et al.*, 2012). Although, correlation between genotypes, seed colour, growing conditions and nutritional analysis has never been attempted. The seed is a useful ingredient in cooking as it can be eaten as a boiled or fried snack, and milled into flour (Goli, 1995). Despite its 'balanced' macronutrient composition, bambara groundnut contains some anti-nutritional factors such as tannins and trypsin inhibitors (Barimalaa and Anoghalu, 1997). A study conducted by Ijarotimi and Ruth (2009) showed that fermentation has significant effects in decreasing the anti-nutritional factors; oxalate, tannic acid, phytic and trypsin.

TABLE 1. Macronutrient status of Bambara groundnut in comparison with some more popular legumes

Nutritional values	Bambara groundnut	Soybean	Phaseolus bean	Cowpea	Faba bean	Chickpea
Calories	390	416	343	333	341	364
Protein (%)	21.8	36.5	23.8	23.6	26.1	19.3
Carbohydrate (%)	61.9	30.2	59.6	60	58.3	60.6
Fat (%)	6.6	19.9	2.1	0.8	5.7	6

Source: (Hillocks *et al.*, 2012)

TABLE 2. Amino acid content (mg/100 gm) of raw Bambara groundnut

Amino acid	Raw Bambara groundnut
Lysine	2.8
Histidine	2.4
Arginine	4.9
Aspartic Acid	5.6
Threonine	2.6
Glutamic Acid	17
Glycine	3.3
Alanine	3.9
Cystine	0.7
Methionine	2.7
Isoleucine	3.9
Leucine	6.9
Tyrosine	3.4
Phenylalanine	4.8

*Values are mean of duplicate samples

Source: (Mazahib *et al.*, 2013)

Challenges towards research and development in bambara groundnut. Being classed as an underutilised crop, bambara groundnut faces several challenges towards its research and development. Bambara groundnut is still an underutilised crop mainly because (i) it currently has limited economic potential outside its areas of cultivation (Azam-Ali *et al.*, 2001); (ii) lack of appropriate processing techniques to overcome hard-to-cook effects (Mazahib *et al.*, 2013); (iii) absence of functioning value chains (Hillocks *et al.*, 2012); (iv) there is very little information and knowledge base on neglected plant species in terms genome information and germplasm collection (Azam-Ali *et al.*, 2001); (v) biological issues such as photoperiod sensitivity to reproductive development and pod-filling, which effects the geographical range/time of planting and yield stability; and (vii) a lack of mechanisation for crop mechanisation (e.g., seed sowing) as well as machinery for post-harvest (e.g., pod-shelling). Furthermore, bambara groundnut is still grown as landraces and its yield can be unstable and unpredictable at different geographical regions. While being adapted to their current environment, landraces may not contain the optimal combination of traits (Massawe *et al.*, 2005). Development of improved varieties of bambara groundnut, through

controlled and coordinated multi-locational breeding programmes, is vital to harness the potential of the crop (Aliyu *et al.*, 2015). The International Institute for Tropical Agriculture (IITA) currently holds the largest *ex situ* collection, with 2055 accessions (as of January 2015) of bambara groundnut. This crop could be a potential exemplar for other underutilised crops. **Breeding/Molecular perspectives.** Historically, genomic and molecular genetic analysis has been focused towards major species, but now with the advancement in high-throughput sequencing technologies, such as NGS, and the reduction in their costs and also the availability of bioinformatic tools (Table 3), it is becoming possible to transfer information to crops and related-species. With the availability of genomic resources and the completion of reference genome sequences of legume crops, such as *Medicago truncatula* (Young and Udvardi, 2009), Common bean (Schmutz *et al.*, 2014), Soybean (*Glycine max*) (Schmutz *et al.*, 2010) and Cowpea (Muñoz-Amatriaín *et al.*, 2016), it is now possible to dissect information and transfer genomic and transcriptomic data to other legume crops such as bambara groundnut. For example, the USEARCH sequence analysis tool can be used for comparing sequences between closely related species (Ward and Moreno-Hagelsieb, 2014), enabling advances in genetic marker development, location of orthologues and decoding of the genetic mechanism and pathways involved in drought tolerance in less studied crops, such as bambara groundnut through genomic and transcriptomic comparative analysis.

Drought stress in bambara groundnut

Drought stress tolerance mechanisms. Drought stress is one of the major abiotic stresses that inhibits proper plant growth and crop productivity. Drought stress is defined as stress that is caused by inadequate soil moisture to meet the needs of a particular crop at a particular time (National Drought Mitigation Center, n.d.). Drought tolerant plants respond to drought stress *via* a series of different mechanisms. They are divided into three groups namely (i) drought escape, (ii) drought avoidance, and (iii) drought

TABLE 3. Bioinformatic tools and databases available for genomic/transcriptomic research

Name	Description	URL/Source
Tools		
Genome Workbench	An integrated application for viewing and analyzing sequence data.	http://www.ncbi.nlm.nih.gov/tools/gbench/
MISA	Allows identification and localization of perfect microsatellites	http://pgrc.ipk-gatersleben.de/misa/
Trinity	Tool for RNA-seq <i>de novo</i> assembly	https://github.com/trinitymaseq/trinitymaseq/wiki
ArrayExpressHTS	R-based pipeline for RNA-seq data analysis	https://bioconductor.org/packages/devel/bioc/html/ArrayExpressHTS.html
USEARCH	Unique sequence analysis tool	http://drive5.com/usearch/
Assembly to Assembly Comparison (ATAC)	For comparative mapping between two genome assemblies, or between two different genomes	http://seqanswers.com/wiki/ATAC
Atlas-SNP2	SNP detection tool developed for RNA-seq platforms	https://sourceforge.net/p/atlas2/wiki/Atlas-SNP/
BLAST Ring Image Generator (BRIG)	Used for comparative analysis between large number of genomes	http://brig.sourceforge.net/
EdgeR	R package for differential expression analysis for RNA-seq data	https://bioconductor.org/packages/release/bioc/html/edgeR.html
solQTL	Tool for analysis and visualization of quantitative trait loci (QTL).	https://solgenomics.net/search/phenotypes/qtl
TriClust	Tool for cross-species analysis of gene regulation	http://www.baskent.edu.tr/~hogul/triclust/
Databases		
ArrayStar	Database that holds collection tools for microarray analysis	http://www.arraystar.com/
Bedtools	Database for comparing large set of genomic features	http://bedtools.readthedocs.io/en/latest/
Bionumerics	Integrated analysis of all major applications in Bioinformatics: 1D electrophoresis gels, all kinds of chromatographic and spectrometric profiles, phenotype characters, microarrays, and sequences	http://www.applied-maths.com/bionumerics

TABLE 3. Contd.

Name/Tools	Description	URL/SourcePhytozome
Comparative platform for green plant genomics	https://phytozome.jgi.doe.gov/pz/portal.html	
Ensembl Genomes	Genome-scale data for plants, bacteria, fungi, protists and invertebrate metazoa.	http://ensemblgenomes.org/
Legume Information System (LIS)	Genomics database for the legume family	http://legumeinfo.org/
Legume Federation	A consortium of scientists working to support robust agriculture	http://legumefederation.org/
LegumelP	Integrative platform to study gene function and gene evolution in legumes	http://plantgrm.noble.org/LegumelP/
Gene Expression Omnibus (GEO) BLAST	Tool for aligning a query sequence (nucleotide or protein) to GeneBank sequences included on microarray or SAGE platforms in the GEO database	http://www.ncbi.nlm.nih.gov/geo/

tolerance. Drought escape is described as the ability of plants to complete their growth cycle and reach maturity before drought-stress develops to damaging levels (Kooyers, 2015). Drought avoidance is demonstrated by crop species, which are able to maintain high water potential in the plant by minimising water loss and maximising water uptake under drought conditions, as seen in Siratro (*Macroptiliumatro purpureum*), a tropical legume (Ludlow, 1989) and chickpea (Gaur *et al.*, 2008). Mechanisms of avoidance include improved root traits, for greater extraction of soil moisture, decreased stomatal conductance, decreased radiation absorption and decreased leaf area for minimal water loss (Harb *et al.*, 2010). Drought tolerance allows plants to survive through water-use efficiency, i.e., performing all biological, molecular and cellular functions with minimal water. Such mechanisms are seen in a range of leguminous species, including mung bean (*Vigna radiata*) (Ocampo and Robles, 2000) and pigeon pea (*Cajanus cajan*) (Subbarao *et al.*, 2000). Plants with drought tolerance mechanisms are able to maintain their cell turgor through osmotic adjustment, which in turn will contribute to maintaining stomatal opening, leaf expansion and photosynthesis throughout the drought period (Collinson *et al.*, 1997). Several studies have reported differential expression of genes under drought stress in major crops (Table 4). Even though a large number of drought-related genes have been identified in plants, their stability of trait expression under different stress conditions is a major concern.

Molecular and physiological effects of drought stress on plants. Drought stress can cause cellular, physiological and morphological changes in the plant, for instance, a reduction in photosynthesis, leaf area and final yield in groundnut (Collino *et al.*, 2001), cowpea (Anyia and Herzog, 2004) and chickpea (Singh, 1991). In pea (*Pisum sativum*), germination and early seedling growth were reported to be influenced by drought (Okcu *et al.*, 2005). Reduction in the number of grains, grain yield, shoot dry weight and harvest index were observed in wheat (*T. dicoccoides*) when it was subjected to drought stress (Gupta *et al.*, 2001). Drought stress can affect crop growth at any developmental stage

TABLE 4. Stress-responsive genes contributing to drought tolerance in plants

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
AP2/ERF Transcription Factor <i>al.</i> ,	DREB1A (CBF)	<i>A. thaliana</i>	Activated expression of genes involved in stress tolerance (rd29A)	Signaling cascade and transcriptional regulation	ABA independent	(Kasunga <i>et al.</i> , 1999; Liu <i>et al.</i> , 1998)
AP2/ERF Transcription Factor	OsDREB1A	<i>O. sativa</i>	Survivability	Signaling cascade and transcriptional regulation	ABA independent	(Dubouzet <i>et al.</i> , 2003)
AP2/ERF Transcription Factor	CBF1 (DREB 1B)	<i>Solanum lycopersicum</i>	Activated expression of genes, catalase 1 coupled with decreased accumulation of H ₂ O ₂	Signaling cascade and transcriptional regulation	ABA independent	(Hsieh <i>et al.</i> , 2002)
AP2/ERF Transcription Factor	HvCBF4	<i>H. vulgare</i>	Survivability	Signaling cascade and transcriptional regulation	ABA responsive	(Oh <i>et al.</i> , 2007)
AP2/ERF Transcription Factor	OsDREB1F	<i>O. sativa</i>	Survivability	Signaling cascade and transcriptional regulation	ABA independent	(Wang <i>et al.</i> , n.d.)
AP2/ERF Transcription Factor	OsDREB1G, -2B	<i>O. sativa</i>	Survivability	Signaling cascade and transcriptional regulation	ABA independent	(Chen <i>et al.</i> , 2008)
AP2/ERF Transcription Factor	OSDREB2A	<i>O. sativa</i>	Survivability	Signaling cascade and transcriptional regulation	ABA independent	(Cui <i>et al.</i> , 2011)
AP2/ERF Transcription Factor	HARDY	<i>A. thaliana</i>	Survivability, WUE, photosynthesis	Signaling cascade and transcriptional regulation	ABA independent	(Karaba <i>et al.</i> , 2007)
AP2/ERF Transcription Factor	TaDREB2, -3	<i>T. aestivum</i>	Multiple	Signaling cascade and transcriptional regulation	ABA independent	(Morran <i>et al.</i> , 2011)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
AP2/ERF Transcription Factor	CBF4	<i>A. thaliana</i>	Activated expression of genes involved in stress tolerance	Drought avoidance	ABA responsive	(Haake <i>et al.</i> , 2002)
bZIP Transcription Factor	OsbZIP23	<i>O. sativa</i>	Relative yield	Signaling cascade and transcriptional regulation	ABA responsive	(Xiang <i>et al.</i> , 2008)
bZIP Transcription Factor	OsbZIP46	<i>O. sativa</i>	Survivability, relative yield	Signaling cascade and transcriptional regulation	ABA responsive	(Tang <i>et al.</i> , 2012)
bZIP Transcription Factor	OsbZIP72	<i>O. sativa</i>	Survivability	Signaling cascade and transcriptional regulation	ABA responsive	(Lu <i>et al.</i> , 2008)
bZIP Transcription Factor	SIAREB1	<i>S. lycopersicum</i>	Multiple	Signaling cascade and transcriptional regulation	ABA responsive	(Orellana <i>et al.</i> , 2010)
bZIP Transcription Factor	ABF3/ABF4	<i>A. thaliana</i>	Reduced transpiration and better survival under drought stress. Growth arrest	Signaling cascade and transcriptional regulation	ABA responsive	(Kang, 2002)
NAC	SNAC1	<i>O. sativa</i>	Survivability, seed setting rate	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Hu <i>et al.</i> , 2006)
NAC	OsNAC9	<i>O. sativa</i>	Multiple	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Redillas <i>et al.</i> , 2012)
NAC	OsNAC10	<i>O. sativa</i>	Multiple	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Jeong <i>et al.</i> , 2010)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
NAC	OsNAC5	<i>O. sativa</i>	Multiple	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Jeong <i>et al.</i> , 2013)
NAC	OsNAC6	<i>O. sativa</i>	Survivability	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Nakashima <i>et al.</i> , 2007)
NAC	SNAC1	<i>O. sativa</i>	RWC, chlorophyll content	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Saad <i>et al.</i> , 2013)
NAC	TaNAC69	<i>T. aestivum</i>	Multiple	Drought avoidance, signaling cascade and transcriptional regulation	ABA responsive	(Xue <i>et al.</i> , 2011)
Zinc Finger	DST		Survivability	Drought tolerance, signaling cascade and transcriptional regulation		(Huang <i>et al.</i> , 2009)
Zinc Finger	ZFP252	<i>O. sativa</i>	Survivability	Drought tolerance, signaling cascade and transcriptional regulation		(Xu <i>et al.</i> , 2008)
Zinc Finger	Zat10	<i>A. thaliana</i>	Yield, seed setting rate	Drought tolerance, signaling cascade and transcriptional regulation		(Xiao <i>et al.</i> , 2009)
Zinc Finger	OsMYB2	<i>O. sativa</i>	Survivability	Drought tolerance, signaling cascade and transcriptional regulation		(Yang <i>et al.</i> , 2012)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
Zinc Finger	TaPIMP1	<i>T. aestivum</i>	Water loss, proline content	Drought tolerance, signaling cascade and transcriptional regulation		(Zhang <i>et al.</i> , 2012)
Zinc Finger	StMYB1R-1	<i>S. tuberosum</i>	Multiple	Drought tolerance, signaling cascade and transcriptional regulation		(Shin <i>et al.</i> , 2011)
Zinc Finger	OsWRKY11	<i>O. sativa</i>	Survivability	Drought tolerance, signaling cascade and transcriptional regulation		(Wu <i>et al.</i> , 2009)
Zinc Finger	OsWRKY30	<i>O. sativa</i>	Survivability	Drought tolerance, signaling cascade and transcriptional regulation		(Shen <i>et al.</i> , 2012)
Zinc Finger	ZPT2 - 3, CpMYB10	<i>Petunia</i>	Better survival rate during drought stress	Drought tolerance, signaling cascade and transcriptional regulation		(Sugano <i>et al.</i> , 2003)
Osmotic Adjustment	P5CS (Pyrroline-5-carboxylate synthase)	<i>O. sativa</i>	Increase in biomass accumulation	Drought tolerance		(Zhu <i>et al.</i> , 1998)
Osmotic Adjustment	SacB	<i>Beta vulgaris</i>	Better dry weight accumulation	Drought tolerance		(Pilon-Smits <i>et al.</i> , 1999)
Osmotic Adjustment	TPS (Trehalose-6-phosphate synthetase)	<i>N. tabacum</i>	Delay in withering or enhanced moisture retention capacity	Drought tolerance		(Holmström <i>et al.</i> , 1996)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
Osmotic Adjustment	IMT1 (myo-Inositol-O-methyl transferase)	<i>Tobacco</i>	Less inhibition in photosynthetic rate; better recovery from stress	Drought tolerance		(Sheveleva <i>et al.</i> , 1997)
Osmotic Adjustment	Trehalose-6-phosphate synthetase	<i>Tobacco</i>	Increased leaf area, better photosynthetic activity and better RWC	Drought tolerance		(Pilon-Smits <i>et al.</i> , 1999)
Osmotic Adjustment	Trehalose-6-phosphate synthetase	<i>O. sativa</i>	Better plant growth and less photooxidative damage	Drought tolerance		(Garg <i>et al.</i> , 2002)
Osmotic Adjustment	TPS and TPP	<i>O. sativa</i>	Better growth performance and photosynthetic capacity	Drought tolerance		(Jang <i>et al.</i> , 2003)
Osmotic Adjustment	AtPLC1	<i>A. thaliana</i>		Drought tolerance		(Hirayama <i>et al.</i> , 1995)
Osmotic Adjustment	OsTPS1	<i>O. sativa</i>	Survivability	Drought tolerance		(Li <i>et al.</i> , 2011)
Osmotic Adjustment	mtlD	<i>E. coli</i>	Multiple	Drought tolerance		(Abebe <i>et al.</i> , 2003)
ROS Scavenging	OsSRO1c	<i>O. sativa</i>	Multiple	Drought tolerance	ABA responsive	(You <i>et al.</i> , 2012)
ROS Scavenging	MnSOD (superoxide dismutase)	<i>Alfalfa</i>	Better photosynthetic efficiency, yield and survival rate	Drought tolerance	ABA responsive	(McKersie <i>et al.</i> , 1996)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
ROS Scavenging	MsALR (Aldose/aldehyde reductase)	<i>Alfalfa</i>	Decreased lipid peroxidation and better photosynthetic activity	Drought tolerance	ABA responsive	(Oberschall <i>et al.</i> , 2000)
ROS Scavenging	AtALDH3 (Aldehyde dehydrogenase)	<i>A. thaliana</i>	decreased lipid peroxidation	Drought tolerance	ABA responsive	(Sunkar <i>et al.</i> , 2003)
ROS Scavenging	Ascorbate peroxidase	<i>N. tabacum</i>	Better photosynthetic capacity under water stress	Drought tolerance	ABA responsive	(Hamid Badawi <i>et al.</i> , 2004)
Protein Kinase	OsCDPK	<i>O. sativa</i>	Enhanced levels of stress-responsive genes, rab16A, SalT, and wsi18. Stomatal movement	Signaling cascade and transcriptional regulation	ABA responsive	(Saijo <i>et al.</i> , 2000)
Protein Kinase	DSM1	<i>O. sativa</i>	Plant growth.	Signaling cascade and transcriptional regulation	ABA responsive	(Ning <i>et al.</i> , 2010)
Protein Kinase	OsSIK1	<i>O. sativa</i>	Survivability.	Signaling cascade and transcriptional regulation	ABA responsive	(Ouyang <i>et al.</i> , 2010)
Protein Degradation	OsDIS1	<i>O. sativa</i>	Survivability			(Ning <i>et al.</i> , 2011)
Protein Degradation	OsDSG1	<i>O. sativa</i>	Fresh weight			(Park <i>et al.</i> , 2010)
Protein Degradation	OsSDIR1	<i>O. sativa</i>	Survivability			(Gao <i>et al.</i> , 2011)
Protein Degradation	OsRDCP1	<i>O. sativa</i>	Survivability			(Bae <i>et al.</i> , 2011)

TABLE 4. Contd.

Functional category	Gene	Species	Parameters evaluated	Mechanisms	Pathways	References
Protein Modification	SQS1	<i>O. sativa</i>	Survivability, relative yield			(Manavalan <i>et al.</i> , 2012)
Nuclear Proteins	OsSKIPa	<i>O. sativa</i>	Survivability, yield	Drought tolerance Transcriptional co-regulator		(Hou <i>et al.</i> , 2009)
Nuclear Proteins	OsRIP18	<i>O. sativa</i>	Survivability	Drought tolerance Transcriptional co-regulator		(Jiang <i>et al.</i> , 2012)
Metabolism Of Aba	DSM2	<i>O. sativa</i>	Survivability, seed setting rate			(Du <i>et al.</i> , 2010)
Metabolism of Other Hormones	IPT	<i>A. tumefaciens</i>	Yield, biomass			(PELEG <i>et al.</i> , 2009)
Dehydrin/LEA	OsLEA3-1	<i>O. sativa</i>	Yield, seed setting rate	Drought tolerance	ABA responsive	(Xiao <i>et al.</i> , 2007)
Dehydrin/LEA	OsLEA3-2	<i>O. sativa</i>	Survivability, grains per spike	Drought tolerance	ABA responsive	(Duan and Cai, 2012)
Dehydrin/LEA	HVA1	<i>H. vulgare</i>	Plant growth, survivability, RWC	Drought tolerance	ABA responsive	(Babu <i>et al.</i> , 2004)
Transporter	AtNHX1	<i>A. thaliana</i>	seed setting rate	Drought tolerance		(Xiao <i>et al.</i> , 2009)
Transporter	betA, TsVP	<i>E. coli</i>	Mutiple	Drought tolerance		(Wei <i>et al.</i> , 2011)
Amino Acid Metabolism	OsOAT	<i>O. sativa</i>	Survivability, relative seed setting rate			(You <i>et al.</i> , 2012)

including, the vegetative, reproductive and grain filling stages at varying degrees depending on the species (Blair *et al.*, 2010). In soybean, the loss of seed yield was reported to be maximal when drought appeared during anthesis and the early reproductive stages (Liu *et al.*, 2003; Eslami *et al.*, 2010). As water resources available for agriculture are expected to decrease and becoming unpredictable due to climate change, the need to adopt and enhance drought-resistant in plants is essential to help to produce enough food for the ever increasing world population, and maintain environmental resilience in agriculture. For example, advanced lines BAT477 and SEA5 that are highly drought tolerant have been identified in common bean (Singh *et al.*, 2001; Teran and Singh 2002). Furthermore, Budak *et al.* (2013) reported the introgression of wild emmer wheat, which is highly drought tolerant, into modern wheat cultivars in order to obtain drought related candidate genes for breeding purpose.

Effect of drought stress on bambara groundnut.

For bambara groundnut, several studies have been carried out to investigate the response to drought stress. Under drought stress, bambara groundnut landrace AS-17 showed paraheliotropic properties, in which the stressed plants had leaflet angles parallel to the incident radiation, leading to less transpiratory water loss due to the lower leaf temperature that resulted from decreased light interception (Stadler, 2009). From the results of Mabhaudhi *et al.*, (2013), bambara groundnut was observed to have drought escape mechanisms where, under drought stress, it had a shortened vegetative growth period, early flowering, reduced reproductive stage and early maturity in order to minimise the adverse effect of drought on plant development. Higher root dry weight was reported when bambara groundnut landrace, Burkina (originally from Burkina Faso), was subjected to drought (Berchie, 2012). Denser and deeper root growth will allow the plant to utilise more soil moisture under drought stress. Stomatal closure plays an important role in regulating transpiration and improve plant water status over the drought stress period. Stomatal closure has been recognised as a universal response to drought stress in many species, such as rice

(*oryza sativa*) (Huang *et al.*, 2009), maize (*Zea mays*) (Benešová *et al.*, 2012) and has been reported for bambara groundnut (Collinson *et al.*, 1997; Vurayai *et al.*, 2011). Accumulation of proline was observed in bambara groundnut under drought stress which plays a vital role in osmotic adjustment (Collinson *et al.*, 1997). Furthermore, (Vurayai *et al.*, 2011) stated that reduced leaf area in drought-stressed bambara groundnut plants due to turgor reduction within expanding cells is common and is one of the earliest physiological responses to water stress. Bambara groundnut is more vulnerable to drought during the pod filling stage, followed by the flowering stage and then the vegetative stage, as plants stressed at the pod filling stage failed to fully recover their relative water content and chlorophyll fluorescence after irrigation was resumed (Vurayai *et al.*, 2011).

Understanding and optimising the responses of bambara groundnut under drought is of central importance in order to identify the key features of the crop which need breeding attention. Drought experiments in bambara groundnut will help to identify novel drought-related genes which could be of great importance to understand biochemical and physiological behaviour of this plant during drought stress.

In an effort to generate drought transcriptomic data for this crop at relatively low cost, NGS technology can be used directly to develop molecular markers by generating transcriptomes (ESTs) which will then be used to identify candidate genes responsible for the crop's response to drought stress. Additional molecular markers can be discovered from translational genomics-based approaches including investigating known gene regulatory networks involved in drought stress response and tolerance in other species. For example, with the help of genomics, transcriptional regulatory networks of drought stress signals were identified in range of species (Shinozaki and Yamaguchi-Shinozaki, 2006), which will help in genomic study of less studied crops, such as bambara groundnut. Use of molecular markers for an agronomically important trait such as drought in underutilised crops, in this example bambara groundnut, will assist the integration of desirable alleles into specific genotypes that will contribute

to improvement of breeding lines and the development of drought tolerant cultivars.

Use of advanced genomics and transcriptomics for research and development in bambara groundnut

Advancement in crop genome sequencing and analysis. Significant progress in sequencing technologies have speeded up the time and lowered the cost per base pair, allowing a step change in access to crop genomes compared to the previous era of Sanger-based sequencing. Before the invention of next generation sequencing technologies, Sanger sequencing of bacterial artificial chromosome (BAC)-based physical maps was the main approach for genome sequencing of species, such as rice, maize and poplar (*Populus tremula*) (Schnable *et al.*, 2009). Though physical maps of BACs provided a good template for completing gaps and correcting sequencing errors, the genome coverage of physical maps was non-representative due to cloning bias and was relatively labour intensive. With the availability and advancement of next generation sequencing, it is possible to sequence large volumes of DNA faster, and with better genome coverage (Metzker, 2010). In 2010, the African Orphan Crop Consortium (AOCC) was launched with the aim to sequence 101 indigenous African crops. Bambara groundnut is one of the target species for sequencing, with the genome sequence generated from a variety from Zimbabwe (Mana), and resequencing will be performed on 100 genotypes developed over the years by multiple research organisations. The draft genome of bambara groundnut is expected to be published in the third quarter of 2016.

Molecular marker systems and breeding for drought resistance using *omic* technologies

Marker-assisted selection from major species to bambara groundnut. With the help of advanced genomic and transcriptomic data, breeders can have access to putative gene function, gene content, copy number variation between varieties, precise genomic positions and identification of both natural and induced variation in germplasm collections. In addition, promoter sequences

allows epigenetic analysis and expression levels to be monitored in different tissues or environments and in specific genetic backgrounds using NGS and microarray technologies (Bevan and Uauy, 2013). The significant reduction in cost and increased accessibility of *omic* technologies (Shendure and Lieberman Aiden, 2012) has made genome-wide analysis of less studied crops possible. The availability of molecular markers and genetic linkage maps in many plant species, such as *Medicago truncatula* (Thoquet *et al.*, 2002), common bean (Freyre *et al.*, 1998), soybean (Song *et al.*, 2004) and cowpea (Menendez *et al.*, 1997) have made it possible to dissect complex traits into individual quantitative trait loci (QTL), with sequencing and annotation of large genomics DNA fragments. Marker-assisted breeding approaches will help in identifying important agronomic traits corresponding to various biotic and abiotic stresses (McCouch *et al.*, 2002). Sequence data derived from *medicago truncatula*, soybean, cowpea and common bean can be used to develop cross-species simple sequence repeat (SSR) markers. For example, *Medicago truncatula* based SSRs provided genetic markers for linkage mapping in alfalfa (*Medicago sativa*) (Sledge *et al.*, 2005) and more distantly related crop legumes (Gutierrez *et al.*, 2005; Zhang *et al.*, 2007). This approach can be applied in bambara groundnut, where a closely related legume crop sequence data, such as common bean, mung bean or soybean can help in identifying molecular markers for the traits of interest. Furthermore, with the help of genome sequencing of major legume crops targeting genes for the specific traits of interest in crop relatives, such as drought resistance, is possible. In this approach, phenotypes of interest in crop relatives are mapped and characterised against major crops using the available genomic resources (Fig. 1) (Young and Udvardi, 2009). This strategy should be applicable to most closely related legume crops and can be applied in bambara groundnut. It was successful in *Medicago truncatula*, where phenotypic mapping was performed in *Medicago truncatula* ranging from disease resistance genes to QTL for morphology. Resistance against *Colletotrichum trifolii* (anthracnose) (*Ameline-*

Torregrosa et al., 2007; Yang *et al.*, 2008), *Phoma medicagnis* (black stem and leaf spot) (Kamphuis *et al.*, 2008), and *Erysiphe pisi* (powdery mildew) (Ameline-Torregrosa *et al.*, 2007) have all been mapped in *Medicago truncatula*. The cloned RCT1 gene found in *Medicago truncatula* for resistance to anthracnose has been shown to function in alfalfa (Yang *et al.*, 2008)

With the help of genomic and transcriptomic analysis, sequence data derived from major species will help in the development of new molecular markers for drought resistance and gene discovery in underutilised crops, leading to crop improvement. Though MAS has been implemented in many crop breeding programmes, it still at a primitive stage for many minor and underutilised crops. Perhaps one of the biggest challenges many underutilised crops face is the absence of structured genetic resources that allow a powerful dissection of the genetic control of complex traits. Many traits are governed by multiple genes and it is more difficult to understand the underlying genetic control of these quantitative traits, without structured (and preferably immortal) populations and genetic stocks. Additionally, imprecise localisation of the QTLs and instability of QTL between experiments and environments adds to the complexity of this approach for underutilised species (Nelson *et al.*, 2004).

Genomic-assisted breeding for drought resistance. The availability of advanced expression analysis techniques such as NGS, microarrays, real-time PCR, transcriptomics, proteomics and metabolomics platforms have made it possible to carry out extensive gene expression analysis to identify and characterise candidate genes for drought tolerance (Swamy and Kumar 2013) (Table 4). Breeding for drought resistance is challenging as it is a complex trait controlled by many genes, there is limited knowledge of the inheritance mechanisms and the effect of drought is different for every genotype (Shashidhar *et al.*, 2013). Knowledge of the relative values of the alternative alleles at all loci segregating in a population could allow breeders to design a genotype *in silico* (Varshney *et al.*, 2005). Marker-assisted backcrossing approaches and marker-assisted

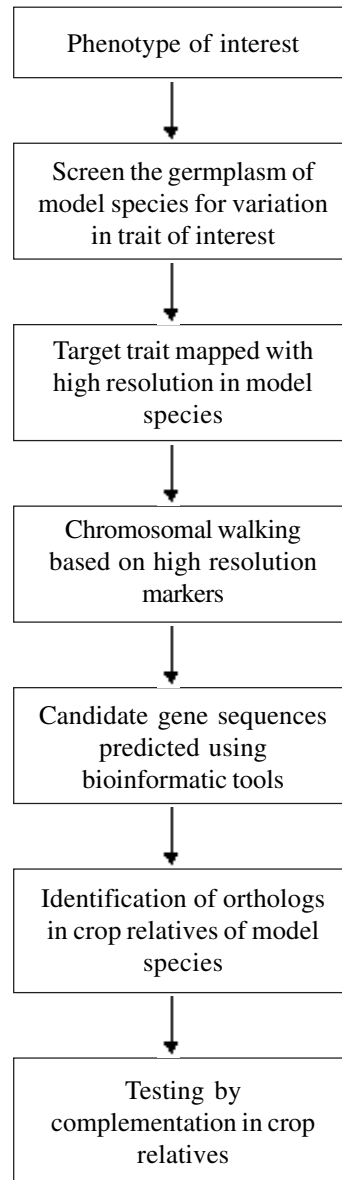


Figure 1. Gene discovery in underutilised species from model species. Key steps listed, where a model species was used to find gene of interest based on genome location (Young and Udvardi, 2009)

recurrent selection have been implemented in legume crops such as chickpea to improve the crop's drought resistance (Thudi *et al.*, 2014). With the recent advances in genomic technologies and the availability of genomic platforms, the cost of genotyping has become much cheaper than phenotyping. Breeding

approaches, such as marker-assisted backcrossing approaches and marker-assisted recurrent selection have recently been complemented by a new approach called genomic selection (GS), which predicts the breeding values (i.e. genomic assisted breeding values; GEBVs) of lines in the next generation based on historical genotyping and phenotyping data (Meuwissen *et al.*, 2001; Morrell *et al.*, 2011). GS has begun to be incorporated into breeding programs for crops, although many questions remain in terms of which crops are most suited and what some of the key parameters for successful deployment are (Eathington *et al.*, 2007). With genome coverage provided by GS, NGS will help in providing estimates of gene expression levels and determination of epigenetic states of genes (Bevan and Uauy, 2013).

To date, there has been no broad screening of the bambara groundnut germplasm under drought stress. Though the crop is known to be drought-tolerant from the physiology point of view, drought tolerance should be linked to comparatively higher growth rates and productivity under water stress conditions. In bambara groundnut, populations were analysed for specific genetic traits of interest which includes genetic mapping of photoperiod response in bi-parental populations after development of single genotype parental lines (Kendabie *et al.*, 2013) and mapping of phenotypic traits associated with domestication syndrome in bambara groundnut and plant morphology (Ahmad *et al.*, 2013; Basu *et al.*, 2007; Chai *et al.*, 2013). The genotypes obtained from crosses in bambara groundnut are important resources to study and optimise for specific traits through breeding programmes (Aliyu *et al.*, 2015). Genomic resources including SSR markers in bambara groundnut were developed and will be employed in identification of QTLs for specific physiological traits relevant for drought tolerance (Beena *et al.*, 2012). Results from Beena *et al.* (2012) showed that there was a significant reduction in the physiological (transpiration, photosynthesis and chlorophyll content) and morphological (leaf area, total biomass) traits under water limiting conditions. The availability of SSR (Beena *et al.*, 2012; Molosiwa *et al.*, 2015) and DArT (Olukolu *et al.*, 2012) markers of

bambara groundnut have made it possible to carry out diversity analysis, mapping of QTLs for various agronomic traits under drought and their use in marker-assisted breeding. Random amplified polymorphic DNA (RAPD) and fluorescence based amplified fragment length polymorphism (AFLP) have been developed for several landraces of bambara groundnut and the study revealed high levels of polymorphism among landraces (Massawe *et al.*, 2002). Genetic linkage maps of bambara groundnut were constructed by combining microsatellite and DArT markers from a 'narrow' and 'wide' cross between bambara groundnut landraces Tiga Necaru x DipC and DipC x VSSP11 to identify marker-trait linkages and to develop the crop through marker-assisted selection by selecting marker allele that is linked to a trait of interest (Basu *et al.*, 2003; Ahmad, 2016). The genetic map created from the Tiga Necaru x DipC cross was based on an intra-sub-specific cross exploits variation within the domesticated landraces gene pool, using an F_3 population of the cross between DipC and Tiga Necaru (Ahmad *et al.*, 2016, 2013; Chai, 2014; Chai *et al.*, 2015). This cross showed variation for agronomic traits of breeding interest. This map will be useful for comparative genomic analysis between the mapping populations in this crop and also between bambara groundnut and other related legume crops (Ahmad *et al.*, 2013). Based on the results from the 'narrow' genetic cross (F_3 generation) and 'wide' genetic cross (F_2 generation) for Tiga Necaru x DipC and DipC x VSSP11, respectively, a candidate marker bgPabg-596774 was identified for the following traits; pod number, node number, pod weight, seed number, seed weight and biomass dry weight which could be used for MAS (Ahmad *et al.*, 2013). Development of the population (Tiga Necaru x DipC and DipC x VSSP11) into full Recombinant Inbred Lines (RILs) (at F_6 generation currently) or the development of Near Isogenic Lines (NILs) could allow the evaluation of the effects of these QTL alone and also the development of heterozygous plants for the QTL region, allowing large-scale fine mapping programme (Ahmad *et al.*, 2013).

Due to the limited understanding of the drought response mechanisms that are active in different germplasm within bambara groundnut,

implementing molecular breeding for drought resistance or selection of candidate genes for gene editing is challenging. Furthermore, high temperature is usually co-incident with drought stress. Therefore, a single gene effect to increase drought resistance is possible, but the combined stress may require a multi-gene transformation strategy that combines several major functional or regulatory genes or a series of genes in a signalling cascade contributing to drought resistance seems promising for improving long term drought resistance in plants (Hu and Xiong, 2014). In addition, combining traditional breeding (such as cross and/or recurrent backcrossing of wild relatives and elite cultivars) will help in building the desired traits for abiotic resistance (Hu and Xiong, 2014) in bambara groundnut.

Comparative genomics and transcriptomics from major species to bambara groundnut. Advances in crop genomics, transcriptomics, molecular and bioinformatic tools have given us an opportunity to understand plant biology in a more unified way and also help transfer information from a major species to minor species (Akpınar *et al.*, 2013). Strong conventional breeding practice is required for successful genomic investment on underutilised crops (Nelson *et al.*, 2004). Exploiting biotechnological tools and translation research from major species to underutilised crops will lead to better results in breeding processes using two main methods (1) translation of technologies, such as marker system approaches based on next generation sequencing (2) translation of actual genetic-trait information from related species based on locational or network analysis (Aliyu *et al.*, 2015).

Comparative genomics. Comparative genomics has been widely used in modern day research as it can provide important information about species whose genomes have not been sequenced by comparing with a known species (Dhanapal, 2012). Rapid advancement in crop genomics has provided a chance to conduct detailed functional and structural comparisons of genes involved in various biological processes among major crops and other plant species. Therefore, comparative genomics using bioinformatic tools can provide an opportunity

for transferring important information from major species to bambara groundnut. Some examples that illustrate comparative genomics in legumes are; (1) Use of barrel medic to map-based clone the RCT gene that confers resistance to multiple races of anthracnose (*Colletotrichum trifolii*) in alfalfa (Yang *et al.*, 2008), (2) Floral regulatory genes identified in *Arabidopsis thaliana* were used to find genes in common bean effecting determinacy (Cannon *et al.*, 2009) and (3) Identification of the gene underlying Mendel's *I* locus, responsible for the trait corresponding to yellow or green colour of seed in grass meadow fescue (Armstead *et al.*, 2007). A candidate gene that plays a critical role in chlorophyll catabolism during plant senescence was found in rice and later its orthologous gene was fine mapped in pea (*Pisum sativum*). Thus traits, genes, tools and species were combined to link this trait and the underlying gene in several models and crops (Armstead *et al.*, 2007). In addition, minor crops can provide good models for a trait and trait analysis absent in major crops (Nelson *et al.*, 2004). For example, if alleles contributing drought tolerance can be found in bambara groundnut, the underlying physiological mechanism and the genes responsible could be useful and can be utilised by MAS to search for alleles within the species of interest or the trait might be transferred through direct gene transfer into major crops

Comparative transcriptomics. Comparative transcriptomics has been tested in bambara groundnut. Comparisons between the bambara groundnut leaf transcriptome and other species has been carried out to identify appropriate cross-species orthologues and gene models for the crop (Mayes *et al.*, 2013). The results showed that soybean had the highest transcript sequence similarity to bambara groundnut than any other species used in the analysis (other species were *Medicago truncatula*, *Vitis vinifera*, *Populus trichocarpa*, *Ricinis communis*, *Arabidopsis lyrata*, *Vigna radiata*) and could potentially be used as a gene model for gene expression profiling in bambara groundnut (Mayes *et al.*, 2013), although the tetraploid nature of soybean adds complications.

Bambara groundnut DNA was hybridised to Arabidopsis ATH1 and *Medicago truncatula* Affymetrix GeneChips for high and low stem number respectively as there is no Affymetrix GeneChip available for bambara groundnut (Chai *et al.*, 2013; Chai *et al.*, 2015). The cross species microarray approach coupled with genetical genomics has been applied on bambara groundnut using the soybean Gene Chip array. The drought experiment conducted by (Chai, 2014) used leaf RNA from an F₅ segregating population derived from a controlled cross of between DipC and Tiga Necaru cross-hybridised to the soybean GeneChip™ from Affymetrix. The results identified 1531 good quality gene expression markers (GEMs) on the basis of differences in the hybridisation signal strength. An expression based genetic map was constructed using 165 GEMs. Significant QTLs were detected using the GEM map for various morphological traits (including internode length, peduncle length, pod number per plant, pod weight per plant, seed number per plant, seed weight per plant, 100-seed weight, shoot dry weight). An XSpecies microarray experiment was conducted in order to identify and detect genes and gene modules associated with low temperature stress responses in bambara groundnut. This found 375 and 659 differentially expressed genes ($p < 0.01$) under the sub-optimal (23°C) and very sub-optimal (18°C) temperatures, respectively. Further, 52 out of top 100 differentially expressed genes were validated using NGS technology generated from the same samples used to generate cross-species microarray data. The results showed >50% similarity between the XSpecies Microarray approach and NGS technology. The identified gene modules could be useful in breeding for low-temperature stress tolerant bambara groundnut varieties (Bonthala *et al.*, 2015). These approaches have the potential to identify polymorphisms between individuals for gene expression analysis and mutation discovery (Mardis, 2008), which will accelerate the generation of markers for specific traits in minor crop species (Chai *et al.*, 2013).

CONCLUSION

Recent developments in genomics and transcriptomics have opened up opportunities to develop data sets for several underutilised species which could facilitate crop improvement. Genomics has led us to gather a wealth of information from the identification of genetic variation, epigenetic states of genes and the potential to measure gene expression with high precision and accuracy. This will not only benefit breeding but also facilitates systematic comparison of gene functions across sequenced genomes which will directly benefit crop improvement. Projects, such as Encyclopedia of DNA elements (ENCODE) (although yet to be implemented in plants) will build a foundation for extracting knowledge of gene function and variation, thus generating new data for the prediction of phenotype from genotype (Bevan and Uauy, 2013). Knowledge gained from integration of gene function into networks, such as controlling flowering time in response to day length and over-wintering will pave the way for crop improvement. These networks have been identified in *Arabidopsis* and Rice, with allelic variation strongly influencing networks outputs. Processes, such as gene duplication and footprints of domestication can be mapped to networks such as flowering time (Yan *et al.*, 2006; Higgins *et al.*, 2010). Improved precision of predicting the phenotype from genotype is possible with the use of ‘systems breeding’ approaches which use diverse genomic information leading to food security and crop improvement (Bevan and Uauy, 2013).

The improvement in genomics and transcriptomics will help in identifying target genes that underlie key agronomic traits related to drought. Molecular markers will be developed using the information gained from the trait of interest which will be later on used for breeding applications. Finding gene targets that are related to various biotic and abiotic stresses will be productive towards the aim of crop improvement, as plant growth is severely effected by stresses, such as drought, cold and salt especially in

marginal physical and economic environments. Molecular analysis of bambara groundnut germplasm using advanced genomic tools will help in the discovery of genes for key agronomic traits. Functional genomic tools, physical maps and the availability of high-throughput and cost-effective genotyping platforms will all contribute towards crop improvement. There are various challenges that have to be looked upon before applying genomics to underutilised crops. Most of the underutilised crops lack large-scale collections of germplasm, although local communities and small-scale farmers do have extensive knowledge that help in the search for genes that are vital for crop improvement. Restriction on flow of germplasm due to intellectual property rights is also one of the major challenges (Bhattacharjee, 2009).

The potential value of underutilised crops (and bambara groundnut) is the part they can play in minimising Africa's challenges of rural development, hunger, malnutrition and gender inequality. Bambara groundnut is resilient and reliable crop that thrives in unsuitable areas which could potentially be unsuitable for peanut, maize, or even sorghum (*Sorghum bicolor*) (Murevanhema and Jideani, 2013). It is mainly reported to be grown by women, therefore, offers a reliable way of empowering women financially, hence improving the lives of their families (National Research Council, 1996). As a legume, it has the ability to fix atmospheric nitrogen either as an intercrop or rotational crop, thus minimising the use of chemical fertilisers. Being a cheap source of soil nitrogen, it can help resource-poor farmers to achieve some added nitrogen for the growth of the main crop.

The Green Revolution only ever partially succeeded in Africa, but has led to a focus on major crops which, in some cases, are been grown in the wrong places and under the wrong agricultural systems for them to ever be truly productive. Investigating the many underutilised crops which exist as a component of climate resilient, low input, agriculture is one way to mitigate the risk of total crop failure. The advent of next generation sequencing has opened up the possibilities for minor crops, allowing both within species analysis and comparative analysis to related species. Access to germplasm is still a

significant issue, but the tools to begin a more context and nutritionally focused agricultural revolution are coming into place.

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