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Final report and project summary

BAMLINK

Molecular, Environmental and Nutritional Evaluation
 of
 Bambara Groundnut (*Vigna subterranea* L. Verdc.) for Food
 Production in Semi-Arid Africa and India



**EU INCO-DEV
 SIXTH FRAMEWORK PROGRAMME**

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-Assessment of Genetic Variability of Bambara groundnut (<i>Vigna subterranea</i> (L.) Verdc) Accessions using Morphological traits and Molecular Markers O. Molosiwa ¹ , S. Basu ¹ , F. Stadler ² , S.Azam-Ali ³ and S.Mayes ¹	108
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EXECUTIVE SUMMARY

Background

Bambara groundnut is an indigenous legume grown mainly for subsistence in much of Africa. It has the potential to contribute to food security in drought-prone regions, especially in much of the semi-arid tropics where rainfall is often insufficient to support the cultivation of other leguminous crops. Since 1992, the European Union (EU) has supported research on Bambara groundnut. The BAMGROW project (1992-96) assessed the agro-ecological potential of the crop and linked field experiments in Tanzania, Botswana and Sierra Leone with experiments and analysis at the University of Nottingham, UK and Wageningen University in the Netherlands. The BAMFOOD project (2000-04) linked molecular, physiological and agronomic studies between partners in Africa (Botswana College of Agriculture, University of Swaziland and Ministry of Agriculture, Water and Rural Development, Namibia) and Europe (University of Nottingham, UK and Technical University of Munich, Germany). In January 2006, a third EU-funded project, BAMLINK, was launched to evaluate the nutritional, ecophysiological and molecular characteristics of Bambara groundnut landraces.

BAMLINK

The ten partners in BAMLINK are:

- P1. Division of Agricultural and Environmental Sciences, University of Nottingham, UK (GBR)
- P2. Dept. of Crop Science and Production, Botswana College of Agriculture, Botswana (BOT)
- P3. Dept. of Agric Sciences, Royal Veterinary and Agricultural University, Denmark (DEN)
- P4. Chair for Plant Breeding, Technische Universität München, Germany (GER)
- P5. Crops Research Institute, Kumasi, Ghana (GHA)
- P6. National Research Centre for Groundnut, Gujarat, India (Guj)
- P7. Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India (KAR)
- P8. Department of Chemistry, University of Namibia, Windhoek, Namibia (NAM)
- P9. Central Arid Zone Research Institute, Rajasthan, India (RAJ)
- P10. National Plant Genetic Resources Centre, Arusha, Tanzania (TAN)

BAMLINK project management involves four Activity Type (AT) Leaders with overall responsibility for integrating workpackages (WP's) and deliverables within their own AT. The Executive Summary includes reports from each AT leader i.e.

- AT1: Dr Sean Mayes, (GBR)
- AT2: Professor Joergen L Christiansen (DEN)
- AT3: Dr Hans Adu-Dapaah (GHA)
- AT4: Professor Abu Sesay (BOT)

Project Extension

Previous BAMLINK reports have described work done by each BAMLINK partner in relation to the Activity Types and work packages described below.

Activity Type	Workpackage	Partners
AT1. Molecular Characterisation	WP1. Genetic Characterisation WP2. Genetic Linkage Mapping	P4, P1, P3, P5, P8 P1, P2, P4, P7
AT2. Ecophysiological Interactions	WP3. Water Use Efficiency WP4. Heat Stress WP5. Cold Stress WP6. Photoperiodic Responses WP8. GXE Interactions and Heritability	P7, P2, P3, P4, P6, P8, P10 P8, P1, P2, P5, P6, P9 P10, P1, P5, P7, P8, P9 P9, P2, P3, P5, P6, P7, P10 P3, P1, P2, P6, P7, P9, P10
AT3. Nutritional/Functional Characterisation	WP7. Nutritional/Functional Characterisation	P5, P1, P2, P6, P7, P8, P10
AT4. End-User Benchmarking	WP9. End-User Benchmarking – Africa WP10. End-User Benchmarking – India	P2, P5, P8, P10 P6, P2, P7, P9

On 7 December 2009, a request was made by the BAMLINK Project Co-ordinator, Professor Sayed Azam-Ali, to the Research Directorate of the European Commission for a no-cost project extension to 63 months from the original duration of 48 months. This request was granted by the EU Commission on 26 February 2010.

Extension of BAMLINK Project – Activities during the extension

period
The Project Extension required the following activities:

1. Restructuring of project around regional hubs to complete work including that contracted to underperforming partners

TAN (P10) and KAR (P7) failed to make a serious and sustained contribution to the project. TAN was formally ejected from the project in August 2009. The removal of this partner meant that the activities and reporting requirements of TAN and, in particular, the leadership of WP5 were reassigned to GBR. Similarly, the poor and incomplete performance of KAR especially with respect to WP3 meant that completion of these activities was assigned to GUJ.

For remaining deliverables to be achieved by the end of Period 5 plus the wider dissemination and impact, it was agreed that remaining BAMLINK activities would be focussed around *three regional hubs*.

- a. GBR (Europe). Co-ordination and leadership, completion of AT1 and contribution to AT2 and linking with DEN (P3) to complete WP8. Completion of final scientific, management and financial reports.

- b. GHA (Africa). Completion of AT3 and AT4, contribution to completion of AT2.

- c. GUJ (India). Contribution to AT4 and AT2 and supply of analysed datasets for AT1 and AT3.

2. Maximising international impact of BAMLINK

BAMLINK is already attracting significant interest from researchers, policy makers and the media who recognise the project as a role model for other underutilised species. It was agreed that maximising impact would be based around three priorities.

a. AQUACROP/BAMGRO

In 2008, FAO released the global AQUACROP model with bambara groundnut as the only underutilised species. AQUACROP workshops have now been held internationally that promote BAMLINK activities and increase project impact. It was agreed that the BAMLINK team would develop BAMGRO within the AQUACROP suite of models to facilitate wider dissemination of project activities and deliverables.

b. Bambara Groundnut Improvement Programme (BAMBREED)

An international improvement programme (BAMBREED) is being developed with partners in Africa and Asia. Ongoing activities between GBR and partners in Indonesia have identified key genetic material and breeding strategies with BAMLINK partners in Africa. It was agreed that further development of the

BAMBREED proposal would be encouraged through existing links within BAMLINK partners and with Indonesia.

Developing a functional delivery system (i.e. a coordinated international breeding programme) is key to translating nearly two decades of research on bambara groundnut funded by the EU into improved varieties in the farmer's field.

3. Links with Global Crops for the Future Centre (CFF)

International Conference on Underutilised Crops.

It was agreed that the project team would contribute to a major conference 'Crops for the Future; beyond food security' to be hosted by GBR (at Nottingham's Malaysian Campus) in 2011.

In 2008, the CFF centre was launched in Malaysia with a global mandate for investigations on promising underutilised food and non-food crops not currently supported by the international agricultural research network. BAMLINK was recognised as a role model for how CFF could investigate other underutilised crops through its global mandate. The research work supported by the EU over the last 18 years has also allowed us to develop a methodology to evaluating new potential crop plants and this will be at the heart of research work for Crops for the Future.

It is no exaggeration to state that without the consistent and long-term support of EU to Nottingham and Partners on bambara groundnut, Nottingham University would not have been in a position to win the co-hosting of Crops for the Future or have been able to persuade the Malaysian government to make the significant and very public commitment of the Crop to the Future Research Centre. For the first time there are dedicated international bodies for the promotion of and research in underutilised crops.

Summary and overview

Outputs and impacts of the BAMLINK project.

BAMLINK has generated new data in all four activity areas, namely:

- Activity 1 Molecular Characterisation
- Activity 2 Ecophysiological Interactions (drought, heat, cold and photoperiod)
- Activity 3 Nutritional/functional Evaluation
- Activity 4 End-user Benchmarking

The current summary report provides an overview of the work undertaken in relation to the original objectives and deliverables. These are presented in tabular form with comments and references to the relevant section in the Annual reports, R1(2006), R2(2007), R3(2008), R4(2009), R5(2010Ex), for example (R1, p21-32). The main activities and findings are summarised below, followed by a detailed analysis of the deliverables for each activity.

AT1 (WPI): Genetic fingerprinting and genetic structure for accessing breeding material.

- A service-based genetic fingerprinting system using DART has been developed.
- 20% of the available accessions from the IITA germplasm collection now have fingerprints.
- Additional samples can be supplied by National programmes and researchers with minimal molecular expertise to produce compatible datasets with previous work. In this way, it is relatively simple for anyone working on this species to know the genetic relationship of their local material to the overall genetic structure of the species.
- 99 SSR markers have been generated for application in quality control, hybrid confirmation, diversity analysis and linkage analysis in breeding and research programmes.
- The likely source of Indonesian accessions of bambara groundnut has been identified.
- A clear genetic split between African accessions north and south of the equator was identified. This genetic structure could have important implications for bambara groundnut breeding and novel variation could be introduced from matched environments in different genetic pools.
- The close relationship between the wild ancestor and cultivated accessions has been confirmed, indicating that it is the true ancestor of the domesticated form.
- An SSR analysis of heterozygosity within bambara groundnut (CE and field) has confirmed strong cleistogamous inbreeding and very limited residual heterozygosity.

AT1(WP2): Genetic Mapping

- The first genetic maps for bambara groundnut have been generated, with a partial integration of the two maps where common markers exist. AFLPs, DART and SSR have all been used.
- The 'wide' map between a domesticated accession and a wild ancestor revealed a number of genetic factors (QTL) for the domestication process. Limited numbers of loci are involved and we conclude that it would be feasible to introgress genes from wild material into domesticated material, if useful traits were present.
- The 'narrow' map between two domesticated accessions revealed a number of genetic factors (QTL) relevant to agriculture.
- For both crosses, genetic markers have been identified which could be developed further for marker-assisted selection.

AT1: (additional work)

- The development of a cross-species approach to detecting markers linked to traits of interest has been evaluated in bambara groundnut (XSpecies)
- A leaf transcriptome based on a coupled molecular/physiology cold stress experiment has been generated. This has been used to:
- 1. Generate 29 polymorphic microsatellites (43% success rate from primary sequence to polymorphic marker.)
- 2. Identify likely intron-exon boundaries for the 17,000 genes in the bambara groundnut leaf, by comparison with *Medicago truncatula* and soybean (*Glycine max*) genomes.
- 3. Overlay the bambara groundnut leaf transcriptome onto the Medicago and soybean genomes, generating a 'pseudo-physical' map for bambara groundnut. We intend to use the genetic maps to integrate this fully in the future by applying a genetical genomics approach.

AT1 (additional work, not EU funded): Progress towards breeding

- The low levels of residual heterozygosity identified through SSR analysis essentially allow us to consider all seed derived from a single plant as genetically identical and as essentially unselected cultivars.
- We have now adopted this as a standard approach for landrace-derived lines and use multiple parental seed derived from single plants to produce large numbers of offspring in controlled crosses.
- We have refined the crossing procedure, such that recent success rates (confirmed by SSR) are 40% (52% of tested putative hybrids, as a number of seed were lost from pod set to maturity; n=42), although pod set from attempted crosses is still relatively low (10%).
- Coupled trait and molecular screening has allowed:
- 1. A glasshouse (UK) and field study (Botswana) that screened 119 accessions from ITA and identified 7 with promise for release in Botswana.
- 2. A CE room and field study (Indonesia) that identified a number of African accessions with potential in Indonesia for release as cultivars and a range of African x Indonesia crosses have been made for further selection.

Overall, the molecular work carried out in BAMLINK has elucidated fundamental aspects of the biology and genetics of this species as well as providing the tools and approaches needed for an applied breeding programme, to move the research knowledge into directly applied action in farmers fields.

AT2 (WP3) Water Use Efficiency

- Experiments by P7 in India have validated the use of Carbon Isotope Discrimination (CID) as a surrogate for WUE – R^2 for instantaneous and cumulative WUE and CID were 0.69 and 0.71, respectively, making it a potentially useful approach for breeding selection.

- Indian partners (P6, P7) have carried out detailed analysis of leaf water relations, proline, sugar and other osmoregulators, the effects of restricted water on root development, epicuticular wax loads. From these analyses a number of landraces showing differential responses for WUE and drought tolerance have been identified for further analysis.

- P4 has taken a Next Generation Sequencing approach to identify genes significantly down or up regulated under non-terminal drought stress, leading to the development of a small microarray representing 132 genes, of which 80 showed significant up or down regulation and were validated in a repeat of the initial experiment, using material from P1 2008 TCRU experiment.

- P4 data analysis provides the basis for initial candidates for WUE/drought tolerance and the early results from P4 are now being retrofitted onto the recently developed leaf transcriptome from the cold stress experiment during the extension period, to allow any common cold/WUE/drought inducible genes to be identified.

- Field experiments in P2 and P5, together with a series of glasshouse experiments in P1 examined the interactions between drought and temperature, with some landraces able to achieve 4.4 t/ha pod yield under rain-fed conditions.

- Glasshouse experiments at P1 revealed a strong positive link between temperature and vegetative development, with a strong negative link between temperature and reproductive development. The potential impairment of this relationship existed with or without irrigation. The potential impairment of reproduction at 33°C has important implications for bambara groundnut and the need for temperature stress tolerance.

- Interestingly, it was also observed that higher temperatures may be reinforcing photoperiod sensitivity in photoperiod partially insensitive material, suggestive of an interplay and integration of environmental signals for reproductive development.
- Genetic differences were observed in a number of experiments across landraces for responses to water stress, based on water relations, stomatal density, chlorophyll traits and also xylem vessel number and length, suggesting a clear adaptive response which varied between genotypes.

AT2 (WP4) Heat Stress

P6 and P7 showed that maintenance of cell membrane stability, chlorophyll stability and thylakoid membrane stability were all confirmed to be important for heat stress tolerance.

- Indian partners proposed a high throughput screening using a Leaf Cell Membrane Thermostability (LCMT) approach to identify heat tolerance material.
- Clear genetic variation among landraces for LCMT was identified in a number of experiments both in African and India.
- There was a significant positive correlation between temperature and seed emergence.
- P3 identified a clear physiological effect of heat stress to be a reinforcement of photoperiodic requirement, with semi-insensitive material shown to become more sensitive at high temperatures.
- P1 and other partners demonstrated that high temperature also leads to decreased pod-set and increased vegetative development in a genotype-dependent way. Whether this is a direct effect of reduced fertility at higher temperatures leading to allocation of resources to vegetative growth or whether it is an intrinsic effect of temperature is unclear.
- The latter two results suggest that there is likely to be a direct interaction between the strength of photoperiod requirement and temperature in bambara groundnut, which is an important finding to consider in the development of photoperiod 'insensitive' material for non-equatorial regions.

AT2 (WP5) Cold Stress

-In response to a series of sowing date experiments by P1 and other partners, it is clear that PSII efficiency declines with temperature.

-Seed emergence decreased significantly with cold temperatures, although genetic variation between landraces does exist.

-A detail CE column experiment at P1 demonstrated the effects of plant development and phenology at low temperature (28°C vs 23°C vs 18°C) in two landraces.

-Landrace-specific effects were seen for both root and shoot effects.

-Molecular analysis of this experiment generated the first comprehensive bambara groundnut leaf transcriptome and allows a detailed analysis of the 2,000 – 3,000 genes which show temperature and/or landrace specific changes.

-Crosses between two landraces shown to exhibit differential responses to low temperatures have been made, but have yet to be analysed.

- A clear reinforcement of photoperiod requirement by high temperatures was observed by P3 in CE work, which suggests lower temperatures reduce the stringency of photoperiod requirement, although this has not been explicitly tested.

AT2 (WP6) Photoperiod

-Six CE room experiments have tested 250 lines for photoperiod requirement, identifying a number of landraces showing different levels of photoperiod sensitivity, including the least sensitive TZA-1505.

-Effects of increasing temperature on these lines suggested that higher temperatures reinforced photoperiod sensitivity.

-In field experiments in Ghana five moderately insensitive landraces were identified

-In experiments in India phenological effects of altered photoperiod were identified and their interaction with temperature evaluated.

-Pot-bound plants (presumably with higher ABA levels) negatively affected both flowering and pod filling.

-Tiga Necaru, also reported to be photoperiod insensitive (although less so than TZA-1505 as shown in direct comparisons) is one parent of a controlled cross and is ready to be evaluated further. Material from this cross is currently planted in three photoperiod regimes (12/14/16hr) in the new FutureCrop tropical glasshouse facility at P1.

- Both planting date experiments and CE experiment show that while there can be a photoperiod effect on flowering, it is the photoperiod effect on podding which has the strongest influence.

-Controlled crosses between the insensitive and sensitive material, to introduce (partial?) photoperiod insensitivity into bambara groundnut lines is underway

AT2 (WP8) G x E Interactions

- Five landraces suitable for growing in western India have been identified and planting times determined. Seed are being multiplied.
- In Gujarat (P6), detailed leaf anatomy traits were correlated to drought resistance and strong genetic determination of a number of traits found.
- The Global release through the FAO of the bambara groundnut version of the AQUACROP model as a development of the BAMGRO-stress model developed here makes bambara groundnut the only underutilised species to have been implemented in the AQUACROP suite of models.
- BAMGRO-stress was developed to include landrace specific parameters and provides one platform for the eventual integration of high level crop models and the underlying molecular and physiological mechanisms determining the trait
- A full BBSRC UK proposal is currently under consideration to link mathematical modelling of canopy development in bambara groundnut with the high level BAMGRO and AQUACROP models.
- GRASP is being developed as an integrated Geospatially-anchored database which will allow agro-ecological matching and climate change scenarios to be run on a range of crops and pests, including bambara groundnut.

AT3 (WP7) Nutritional/Functional Evaluation

- Five partners and 1 subcontractor have produced detailed information on proximate analysis, anti-nutritionals, such as phenols and tyrosin-inhibitors and the effects of different stresses on nutritional composition.
- The effects of different Rhizobium strains suitable for India on nodulation and yield have been extensively investigated.
- Extensive questionnaires for African partners have revealed the preferences of farmers, merchants and processors.
- Current storage methods in Ghana have been surveyed and improvements developed. A number of products (including flours and milk) have been surveyed to indicate preferences, with bambara groundnut comparing favourably to other alternatives, such as soya.
- A reasonably simple processing procedure for bambara groundnut to give High Quality Bambara Flour (HQBF) was found to reduce poor flavour and antinutritional content.
- Pasty products made by partial substitution of wheat with HQBF were positively received by a taste panel, as was milk made from HQBF.

- Composition of seed was found to be a useful supplement to the diet and the nutritional quality of non-seed parts of the plant were found to be good for fodder.
- A method has been developed for 'bamba sauce' as a potential value added product.

AT4 (WP9) End User benchmarking – Africa

- Detailed ideotypes desired by farmers have been defined.
- Perceptions of and reasons for growing bambara groundnut determined in four African countries.

AT4 (WP10) End User benchmarking – India

- Limited seed availability has made widespread testing of consumer acceptance not possible to carry out.
- Farmers have been introduced to the crop in demonstration plots.

Problems encountered - general

Seed supply

The majority of deliverables have been met, although a number of persistent issues were unable to be resolved during the project. The most important of which were difficulties with generating and maintaining seed stocks, compounded by difficulties with importing material into India because of quarantine regulations. The initial plan involved the split-site multiplication by Indian partners (under quarantine conditions) to allow export of seed as needed to Africa, but also to ensure that all core lines were present in India. This did not succeed due to atypical weather at both sites of multiplication. Rather ironically, because of the limited seed available, there was enough seed to begin activities in India with the defined landraces, but not in Africa where import of seed would have been a simpler issue. Because of this problem, not all 6 core landraces were present with all partners at the start of the project. This complicates the GxE analysis. The attempts to re-multiply core material for the various WPs also then delayed the multiplication of the cross material for QTL analysis work.

Release of funds

Problems encountered with the timely release of funds after each report cycle have had a significant effect on the ability of DC partners to maintain research across years, continuously employ staff and to contribute to the overall project in an integrated way. European partners were able to ensure continued funding through forward funding by their own institutions.

Aims of the Final Report

The final report will address the individual deliverables of the project and assess whether they have been met. If they have not been met, then the progress made towards them and the reasons for their not being met will be examined. The deliverables are presented below as originally envisaged. Where modifications have been made during the progress of the project to deliverables, these will be discussed at the appropriate point.

Key:

R1	Bamlink interim report (Jan 2006 – Dec 2006):
R2	Bamlink interim report (Jan 2007 – Dec 2007):
R3	Bamlink interim report (Jan 2008 – Dec 2008):
R4	Bamlink interim report (Jan 2009 – Dec 2009):
R5	Bamlink interim report (Jan 2010 – March 2011):

P1	Division of Agricultural and Environmental Sciences, University of Nottingham, UK (GBR)
P2	Dept. of Crop Science and Production, Botswana College of Agriculture, Botswana (BOT)
P3	Dept. of Agric Sciences, Royal Veterinary and Agricultural University, Denmark (DEN)
P4	Chair for Plant Breeding, Technische Universität of München, Germany (GER)
P5	Crops Research Institute, Kumasi, Ghana (GHA)
P6	National Research Centre for Groundnut, Gujarat, India (GUJ)
P7	Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India (KAR)
P8	Department of Chemistry, University of Namibia, Windhoek, Namibia (NAM)
P9	Central Arid Zone Research Institute, Rajasthan, India (RAJ)
P10	National Plant Genetic Resources Centre, Arusha, Tanzania (TAN)

Deliverables list table and comments ATI Molecular characterisation

Deliverable No ¹	Deliverable title
D1.1	Workshop on diversity array technique held by subcontractor SCI.
D1.1	<p>Not held.</p> <p>Dr Killian from DART Pty Ltd (Canberra) will present a plenary paper at the 2nd International Underutilised Crops conference (27th June – 1st July 2011, Kuala Lumpur, Malaysia) and will hold a meeting with interested parties on 28th June.</p> <p>The meeting was initially not held due to start-up problems and disappointing initial results with the DART Discovery Array (see below). Two attempts to hold the workshop were organised, but for reasons beyond our control neither happened.</p>
D1.2	+100 DART markers included in bambara groundnut map
D1.2	<p>Completed.</p> <p>146 DART markers segregating in the 'wide' mapping population, 211 segregating in the 'narrow' mapping population (R5, p31-39)</p>
D1.3	Genotyping DART array of 300 markers for bambara groundnut standard genome profiling
D1.3	<p>Completed.</p> <p>The initial DART Discovery array was disappointing, with 76 polymorphic loci from 32 genotypes. A sequencing study by P4 suggested that 44 of these sequences were identical, suggesting a repetitive sequence was being captured by the process. (R2, p90-94; D1 SCI)</p> <p>P1, P4 and SCI decided to invest additional resources in a modified second round of discovery and, with SCI, an array of 7680 features were developed (R3, p127-133) Against over 400 genotypes 361 informative markers were identified for profiling from a total of 635 across the entire array (R4, p69-74; D1 and D2 completed, SCI; supply of materials by SCI completed and PhD Thesis from P4; Florian Stadler)</p>
D1.4	Complete genotyping of all used bambara groundnut genotypes in national collections
D1.4	<p>Completed.</p> <p>P5 supplied ten Ghanaian landraces to P4 for DART analysis (R1, p.81)</p> <p>P6 used AFLPs and protein stains to locally develop genotyping for their received landraces (R2, p136-146) P8 applied RAPDs to characterise landraces (R4, p130-136)</p> <p>National material from Partners was requested and samples sent to P4 for growth and supply of DNA to SCI (R4, p69-74) and comparative analysis of SSR vs DART markers as part of submitted PhD Thesis from P1; Odireleng Molosiwa</p>
D1.5	Genotyping and classification of 400 bambara groundnut accessions from IITA genebank

D1.5	Completed. A total of 429 genotypes have been analysed, including multiple samples from single landraces. The vast majority of these are derived from ITA accessions (R4, p69-74)	D1.6
<p>The DART fingerprints are available in an excel format which is searchable, but we plan to integrate the fingerprints into a publicly available database (originally SC3 was to carry out this integration, but the need to multiply cross material, - with some success - lead us to decide it was better to use the money available for adding passports to the accession data was better spent trying to multiply material).</p> <p>With seed funding from F1, we are developing a Geospatial database for crop germplasm "GRASP" and the genetic data from this project is being loaded. A framework version can be seen at http://grasp.nottingham.ac.uk (Appendix 2). The initial version contains data on wheat as an initial test, but we intend to expand the database to include a wide range of crop and pest species. UoN has indicated that it has a long-term commitment to maintaining and updating this database.</p>		D1.6
<p>Essentially completed.</p> <p>The DART fingerprints are available in an excel format which is searchable, but we plan to integrate the fingerprints into a publicly available database (originally SC3 was to carry out this integration, but the need to multiply cross material, - with some success - lead us to decide it was better to use the money available for adding passports to the accession data was better spent trying to multiply material).</p> <p>With seed funding from F1, we are developing a Geospatial database for crop germplasm "GRASP" and the genetic data from this project is being loaded. A framework version can be seen at http://grasp.nottingham.ac.uk (Appendix 2). The initial version contains data on wheat as an initial test, but we intend to expand the database to include a wide range of crop and pest species. UoN has indicated that it has a long-term commitment to maintaining and updating this database.</p>		D1.6

The DART analysis produced a successful and functional DART Array for genetic fingerprinting, diversity analysis and mapping in bambara groundnut. The decision to invest in a second round of array development after the poor results obtained in the first round (similar to results reported in cultivated pigeonpea) was vindicated. A total of 635 polymorphic markers were identified across 429 bambara groundnut accessions. Because of issues of potential repeat clones identified in the original discovery array, we have taken a conservative approach to scoring, only using markers which give different patterns of presence or absence in the material under analysis. This is likely to underestimate genetic diversity and may potentially distort genetic relationships to a small extent. However, an analysis of geographical accession origin suggested that the diversity analysis strongly reflected geographical origin. Levels of polymorphism in the two controlled crosses were usefully high, at 236 and 146 segregating markers per cross. The mapped markers themselves show some tendency to cluster, with the majority of markers showing linkage to at least one other markers.

This is a solid basis for international diversity analysis in bambara groundnut, with samples of DNA being all that individual research groups need to allow the production of DART data. This is then compatible with previous datasets.

A paper describing the development of the DART markers and their application for genetic diversity analysis has been submitted to BMC Genomics and is in review (Stadler et al., manuscript submitted) and a paper comparing DART diversity data with a morphological assessment has also been published (Olukolu et al., 2011). Florian Stadler's PhD Thesis describes the development of the DART array in detail (Technical University of Munich, Germany) and initial results were reported in Mayes et al., 2009.

A paper will be presented by Mr Odireleng Molosiwa on his use of the developed SSR markers to analyse the genetic diversity of 119 accessions of bambara groundnut, including a comparison of DART, morphological and SSR markers in an overlapping set of genotypes to the 2nd International Underutilized Crop Conference (June 27th -

July 1st, 2011, Kuala Lumpur, Malaysia) which will then become a paper in *Acta Horticulturæ*. Mr Molosiwa has now submitted his PhD Thesis.

D1.7	Important genes specifically involved in drought stress reaction isolated	
D1.7	Completed An experiment examining the effect of drought on four landraces of bambara groundnut was carried out by P4 (R1, p73-79). This involved sampling before and after imposition of drought, followed by RNA extraction from leaves for a Roche 454 analysis. This generated a total of 216,022 sequences of 108bp average length. An initial 2,425 clusters (>10 transcripts per cluster) were identified (R2, p95-105; SC2, deliverables 1&2 completed R2, P106). From this P4 produced an analysis of differentially expressed genes and produce a 132, 50-oligonmer based microarray, with the chip being validated using the TCRU experimental material (GBR) (R3, p134-151; R4, p74,75 and PhD Thesis; Florian Stadler, P4). A good quality leaf cDNA library was also created and sub-contract SC2 (Vertis Biotech) completed (R4 period).	
D1.8	Expression patterns of genes of interest evaluated in different genotypes and physiological states	
D1.8	Completed. P4 used the developed microarray to analyses a range of samples for expression patterns of 132 genes that show alteration under drought stress and to validate results using materials from the 2008 TCRU experiment (R3, p134-151). Results are presented in Florian Stadler's Thesis, TUM, Munich and will be written up for a paper after retrofitting of results with the new leaf transcriptome data (R5, p45-48).	

The MPSS analysis of drought response in bambara groundnut established an initial dataset and also a microarray based on selected genes showing up and down regulation under drought stress. The MPSS data was generated early in the development of Roche 454, so average read lengths were short (average of 108bp) which makes good blast hits difficult to obtain in the absence of homologous species sequence. The selection of 132 genes that showed up or down regulation during the CE room experiment allowed the development of a 50-mer oligonucleotide microarray, which was validated using RNA extracted from the GBR TCRU drought experiment in 2008.

The recent development of a leaf transcriptome based on a mixture of Roche 454 and SOLiD sequencing potentially allows us to overlay the previous results onto the full transcript results, where the same genes are expressed (R5, p45-48)

Details of the initial MPSS experiment and the drought responsive genes identified can be found in Florian Stadler's PhD thesis (P4; Technical University of Munich, Germany) and a retrofitting of data is currently underway before submission of a manuscript for publication. Initial results were presented in Mayes et al, 2009.

Initial results of the recently developed transcriptome will be presented by Dr Sean Mayes, as a paper at the 2nd International Underutilised Crops Conference (details as above) and will become a paper in *Acta Horticulturæ*.

D2.1	Development of a high density composite genetic map of bambara groundnut. Markers linked to testa colour, plant habit and any other simple traits segregating in these crosses.
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D2.3	Detailed, separate and multi-environment QTL analysis, leading to markers of potential use in breeding programmes and the presence in each participating country of the same genotypically defined wide cross germplasm for potential selection work in local programmes.
D2.2	<p>Complete</p> <p>Initial P1 library construction using an filter-oligo capture system is described by P1 (R1, p3-17) and for P7 (R1, 168-170). P1 Initial library screening using a Sanger sequencing approach is described (R2, p23-36) and for P7 (R2, p199-200). The deliverable was modified in 2008 in response to relatively low levels of polymorphism discovered in initial sequencing, particularly from P7. The new target was 100 microsatellites polymorphic within cultivated landraces (R3, p12-28). Primers for amplifying fragments developed at P7 were supplied to P1 for repeat screening against a wider set of germplasm (unreported results) and P7 ceased microsatellite development work, due to very low levels of polymorphism within the P7 library.</p> <p>With costs of Sanger sequencing being an issue, an early 454 approach was adopted and validated (R4, p21, 22)</p> <p>99 SSRs have been characterised against 24 genotypes. The average allele number of alleles >4 and a reasonable number of markers have >9 alleles within this germplasm. 29 markers are derived from transcriptomic data, while the remainder are derived from a genomic repeat-enriched library (R5, p24-30). The details of the microsatellite screening and comparison with both DArT and morphological markers are reported in the submitted PhD Thesis by Odireleng Molosiwa (P1). The final protocol yields a recover rate from primer construction to polymorphic microsatellite of 43%.</p>
D2.2	200 microsatellite markers for use in bambara groundnut breeding programmes. Technology enhancement and transfer pathways to allow partners to use this technology
D2.1	<p>Complete</p> <p>AFLP segregation data was generated in the 'wide' cross and initial microsatellites were screened for polymorphism. Only 3 of 13 polymorphic primers segregated in the 'wide' cross (R1, p10). The initial (78 AFLP+3 SSR) 'wide' map was generated and provisionally located a number of simple traits and domestication loci (R2, p34-35). Given the strength of the domestication effects in this cross, it was decided to concentrate on the 'narrow' cross, as more likely to reflect agriculturally useful variation (R3, p11+14). Sufficient material of the narrow cross was generated after two rounds of growth at SC3 (R3, p35-37; R4, p23-24; and essentially completes modified SC3). Material was dispatched to Ghana (P5) and India (P6), but was not grown and assessed due to financial constraints. The two individual maps contain a total of 236 and 225 markers and a partial integration of groups was completed where there were common markers (R5, p31-45)</p> <p>In on-going PhD work we are trialng a Genetical Genomics approach using XSpecies microarrays. If the approach is promising, we will use the approach to map transcript contigs from the 454/SOLiD transcriptome sequencing onto the 'narrow' genetic map. This will be directly comparable with the Medicago physical genome and allow an integration of the bambara groundnut and Medicago genomes. A comparison of linkage blocks should allow us to investigate genome changes between the two species and also begin genome walking, based on the co-linearity.</p>

D2.3	<p>Essentially complete</p> <p>QTL analysis has been completed for both narrow and wide crosses. This was based on trait data from the UK, Indonesia, IITA (Nigeria; SC3) and Swaziland (R5, p39-45). Lines from the cross were supplied to Ghana (P5) and Gujarat (P6). QTL for a wide range of traits have been identified from both crosses, with Domestication traits predominantly in the 'wide' cross and agricultural traits in the 'narrow' cross.</p> <p><i>In further work the narrow cross is being assessed for maturity, photoperiod response and drought tolerance to integrate a number of physiological responses onto the same cross. A Genetical Genomics approach is being investigated which would lead to a major integration of the genetic and 'pseudophysical' maps.</i></p>
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Microsatellite development was initially split between P1 and P7. Both centres created microsatellite-enriched genomic libraries through amplicon capture approaches and began to sequence inserts for PCR screening for polymorphism against the same 24 genotypes. Initial results from P1 suggested polymorphism levels were reasonable. Initial results from P7 suggested very low levels of polymorphism, although their initial use of a short-run acrylamide system may have hidden some degree of variation, through limited resolution of PCR products.

Initial microsatellites from P7 were retested by P1, who confirmed that the levels of polymorphism in microsatellites derived from the P7 library were far lower than those from the P1 library. Similar sized repeats were present in both libraries, so no clear explanation for the difference in the levels of polymorphism between libraries could be identified. On the basis of initial results and the expenditure on conventional sequencing, it was decided to modify the current target of 200 SSR markers to a more reasonable 100 SSR markers polymorphic within cultivated material. The cost of conventional sequencing had also been a major drain on consumable resources, so a Roche 454 approach was adopted for the second round of genomic microsatellite sequence development. This approach proved reasonably successful, although the short sequences generated by the current single run (unassembled) sequences often left little space to design primers. In retrospect, given that there was a reasonable level of repeat amplicons within the sequenced library, it may have been worth attempting to assemble the sequences before primer design. A re-test and reassessment of the current SSR set identified those with robust amplification.

The final 29 polymorphic SSR markers were developed from the draft Roche 454 leaf transcriptome data, focusing on 3' and 5'-UTRs (generally dinucleotide repeats) or coding sequence (generally trinucleotide repeats). Of 68 sequences designed to transcriptome data, 10 sets of designed primers did not amplify a fragment from genomic DNA, 15 gave clear bands, but larger than expected (suggesting primers spanned introns) and the remaining 43 sequences gave clear bands of approximately the correct size. Twenty-nine of 43 were polymorphic in the 24 genotypes. This gives a recovery rate from design to polymorphic marker of 43%, which is the highest observed in the library work and considerably above the generally reported 10% level. Sequencing of the 15 over-sized products would potentially yield new primers and an additional 6-7 functional microsatellites would be predicted.

Two reasonable density genetic maps were generated for mapping and QTL analysis. The wide map was based on a mixture of pre-existing AFLP data (139 loci), DART markers (146 loci) and SSR (19) of which 60%, 96% and 89% of markers appeared in linkage groups. The low level for the AFLP markers is a potential cause for concern and may indicated problems with data quality or scoring. The narrow map consists of a mixture of DART (205 mapped) and SSR (26 mapped). The narrow map spanned 498cM and the wide map spanned 603cM (only including between marker-marker distances). The predicted linkage length would be around 1100cM for the full genome (with 11 chromosomes), giving a predicted coverage of 45% and 55%, respectively. The percentage of markers linked to at least one other marker suggests that coverage is actually probably greater at 87% and 78%, respectively, and the large proportion of AFLP not mapping have already been noted. These are the first two genetic maps for this species and also the first QTL analyses for trait data.

The 'narrow' cross is a domesticated landrace x domesticated landrace cross, while the 'wide' cross is between a wild ancestor x domesticated landrace. The former is expected to give information on genes segregating within cultivated landraces, while the latter is expected to give information on domestication in this species. Markers associated with a number of traits of relevance were identified, including maturity, drought/water use efficiency, plant habit, stem number, specific leaf area, leaf number per plant, testa colour and seed dormancy.

Traits within the wide cross have previously been discussed in relation to the domestication of bambara groundnut (Basu et al, 2007a) and the initial AFLP map presented (Mayes et al, 2009). Initial results of microsatellite development has been previously described (Basu et al, 2007b, Basu et al, 2007c) and discussed further in Mayes et al., (2009).

The results from the recent mapping and QTL analysis will be presented by Nariman Ahmad as an oral paper at the 2nd International Underutilized Crops conference (details as above) and will appear as a paper in *Acta Horticulturae*. Further details on the further integration of the bambara groundnut transcriptome into a pseudo-physical map on the medicago and soybean genomes (as described in *R5*, p46-49) will be presented at the same conference by Dr Sean Mayes.

AT2 Ecophysiological Analysis

Notes: that the year in which field experiments are reported depends upon the timing of the field cycle in relation to the reporting cycle, so may not occur as a single experiment in each report. Results are only reported once.

The WPs for AT2 show some degree of overlap, due to the interaction between the physiological traits under investigation. WP3, in particular, involves heat/cold/WUE and/or drought, so activities/deliverables are sometime also repeated in other WPs where the specific traits are reported.

The recent death of Joergen Christiansen has prevented completion of WP8 D8.2 and D8.3. We aim to complete this analysis as soon as time and expertise permits. This analysis has also been partly addressed through the BAMGRO-stress model and the more recent AQUACROP bambara groundnut model.

WP3: Water Use Efficiency (WUE).

D3.1	<p>Identification of traits associated with drought, WUE and low and high temperature tolerance including the root traits.</p>
D3.1	<p>Completed</p> <p>P1 completed a high and low temperature TCRU experiment on two landraces (R1, p17-53). In a root block experiment P6 studied the effect of 100% field water capacity against 50% field water capacity on a range of water related characters, including CID and identified significant landrace differences, with S19-3 showing higher drought tolerance (R1, p138-p141) P7 analysed 7 landraces for WUE, chlorophyll and photosynthesis with significant genetic variability identified for most traits between landraces (R1, p154-165) P9 evaluated 7 landraces under water deficit conditions, although cold effected establishment (R1, p77-79).</p> <p>P1 completed a second TCRU experiment on low/high temperature, with/out irrigation. P1 reports initial development of BAMGRO-Stress (R2, p38-46) P4 used 4 landraces (2 'tolerant' to drought, 2 'sensitive') and sampled leaf RNA before and after drought. This was used to develop MPSS data for comparison with physiological states (R2, p95-106) P5 planted 5 landraces at a high temperature site (Tono) with drought, agroforestry (Kumasi) and using multiple sowing dates (photoperiod, Wenchai) (R2, p108-125) P6 completed a detailed root block experiment on WUE, including full physiological, root and water relations assessment on 6 landraces (R2, p149-165) P7 directly evaluated CID as a surrogate for WUE and found strong correlation with both instantaneous and season long CID; $R^2 = 0.69$ and 0.71, respectively (R2, 188-193) P8 evaluated a heat stress experiment using 7 landraces (R2, p205-214)</p> <p>P1 reports results of a TCRU experiment at high and low temperatures with applied terminal drought stress (R3, p38-50) P1 describes module development for BAMGRO-stress (R3, p50-56) P2 assessed the effects of irrigated/non-irrigated, temperature and photoperiod through different sowing dates and effects of water stress (R3, p59-74). P3 pot experiments shows restricted root space will eventually restrict canopy growth and prevent pod formation; photoperiod may act by raising ABA levels (R3, p115-123) P4 reports use of MPSS data to generate a 132 gene oligoarray to evaluate gene expression change in leaf during drought stress (R3, p133-151) P5 experiment using 7 landraces, two locations and different planting dates to evaluate heat stress (R3, p152-163) P6 determined genetic variation for a number of water relation traits (R3, p181-199) P7 pot experiment to determine the effect of water deficit on photosynthesis for 5 landraces (R3, p235-244) P8 6 landraces field sown over 4 months with and without irrigation (R3, p251-284) P9 four landrace experiment under drought/irrigation indicated a strong relationship between RWC and SLA (R3, p296-312)</p> <p>P1 Equation development and testing of BAMGRO-Stress (R4, 26-32) P2 planted 6 landraces, irrigated/non over 3 months, evaluating leaf water relative content and water relations (R4, p34-43) P3 progressive and intermittent drought glasshouse experiment; plant water relations (R4, p49-60) P4 reports completion of data analysis for the 132 oligonucleotide array of drought stress inducible genes (R4, p74-75) P5 pot experiment in Controlled Environment on 13 landraces suggests drought brings flowering forward in some landraces (R4, p77-81) P6 pot experiment examining water relations under stress (R4, p94-107)</p> <p>P1 carried out a low temperature Controlled Environment experiment at $18^{\circ}\text{C}/23^{\circ}\text{C}/28^{\circ}\text{C}$ on both leaf and root, with significant genotype and treatment effects (R5, p51-72)</p> <p>Substantial data on WUE/drought, heat and cold effects have been generated by BAMLINK. The specific effects of heat (WP4) and cold (WP5) are reported in the appropriate sections of the deliverables. Based on the experimental data, the model BAMGRO-Stress has been developed and has also been implemented as the first example of an underutilised crop species in the AQUACROP series of crop models developed by the FAO</p>

D3.2	QTL's associated with high WUE or drought tolerance	
D3.2	<p>Completed</p> <p>P1 reports the identification of QTL to CID, as a surrogate to WUE in the wide cross, where transgressive segregation is observed (R5, p39-45). This builds on the clear identification of CID as a useful surrogate for WUE based on direct measurement of WUE/CID by P7(R2, 188-193). P4 has produced a sequencing based (MPSS) analysis of the induction of drought stress in bambara groundnut (R2, p95-106), leading to the development and validation of a 132-gene oligo microarray (R3, p133-151). The analysis from P4 potentially provides an early list of candidate genes for further genetic comparison with identified QTL locations.</p> <p>Initial candidates and also mapping locations have been identified. The value of CID as a surrogate for the more difficult to measure WUE has been confirmed for this species.</p> <p>A Genetic Genomics approach is currently being explored in work at P1, examining the XSpecies-based analysis of undroughted and water-stressed plants in the narrow cross. If feasible, this would allow the simultaneous integration of the bambara groundnut map into the physical map of the medicago genome, the improvement of the map to high density (>2000 markers) and a QTL analysis to localise genetic effects.</p>	D3.3
D3.3	Knowledge on the mechanism of cross tolerance to drought and heat stress	<p>Partially completed</p> <p>Multiple experiments have been carried out looking at drought and heat stress and their combination (particularly in the TCRU at Nottingham). These experiments have lead to BAMGRO-stress, AQUACROP and an understanding of the combined effects in bambara groundnut.</p> <p>A more detailed GXE integration has not been completed, as noted under WP8 deliverables. We hope to be able to complete this once the appropriate expertise is found, although BAMGRO-stress does integrate a lot of the datasets into a direct model</p>

- Experiments by P7 in India have validated the use of Carbon Isotope Discrimination (CID) as a surrogate for WUE – R^2 for instantaneous and cumulative WUE and CID were 0.69 and 0.71, respectively, making it a potentially useful approach for breeding selection.
- Indian partners (P6, P7) have carried out detailed analysis of leaf water relations, proline, sugar and other osmoregulators, the effects of restricted water on root development, epicuticular wax loads. From these analyses a number of landraces showing differential responses for WUE and drought tolerance have been identified for further analysis.
- P4 has taken a Next Generation Sequencing approach to identify genes significantly down or up regulated under non-terminal drought stress, leading to the development of a small microarray representing 132 genes, of which 80 showed significant up or down regulation and were validated in a repeat of the initial experiment, using material from P1 2008 TCRU experiment.
- P4 data analysis provides the basis for initial candidates for WUE/drought tolerance and the early results from P4 are now being refitted onto the recently developed leaf transcriptome from the cold stress experiment during the extension period, to allow any common cold/WUE/drought inducible genes to be identified.

- Field experiments in P2 and P5, together with a series of glasshouse experiments in P1 examined the interactions between drought and temperature, with some landraces able to achieve 4.4 t/ha pod yield under rain-fed conditions.
- Glasshouse experiments at P1 revealed a strong positive link between temperature and vegetative development, with a strong negative link between temperature and reproductive development. The potential impairment of reproduction at 33°C has important implications for bambara groundnut and the need for temperature stress tolerance.
- Interestingly, it was also observed that higher temperatures may be reinforcing photoperiod sensitivity in photoperiod partially insensitive material, suggestive of an interplay and integration of environmental signals for reproductive development.
- Genetic differences were observed in a number of experiments across landraces for responses to water stress, based on water relations, stomatal density, chlorophyll traits and also xylem vessel number and length, suggesting a clear adaptive response which varied between genotypes.

WP4 Heat Stress

D4.1	Understanding the relationship between SLA, thermotolerance and the genetic basis of such traits
D4.1	<p>Completed</p> <p>P1 used the TCRU to evaluate the effects of low and high temperature on two landraces for photosynthetic, phenological and yield traits (R1, p17-53) P8 evaluated the effect of heat stress on composition of 6 landraces (R1, p171-175)</p> <p>Development of BAMGRO-Stress by P1 (R2, p39-48) P5 planted 5 landraces at a high temperature site (Tono) with drought, agroforestry (Kumasi) and using multiple sowing dates (photoperiod, Wench) (R2, p108-125) P8 evaluated a heat stress experiment using 7 landraces (R2, p205-214) P9 used 2 dates (although note limited seed and disease problems) to look for photoperiod effects (R2, p218 – 228)</p> <p>P1 grew 2 landraces in the TCRU to evaluate the effects of high and low temperature (R3, p32-50) P2 assessed the effects of irrigated/non-irrigated, temperature and photoperiod through different sowing dates; cell membrane stability/photocchemical efficiency (R3, p75-93) P5 experiment using 7 landraces, two locations and different planting dates to evaluate heat stress (R3, p152-163) P8 6 landraces field sown over 4 months with and without irrigation (R3, p251-284) P9 leaf heat kill experiment on four landraces (R3, p289-295)</p> <p>P6 pot experiment examining water membrane thermostability (R4, p108-111) P9 field experiment on heat stress effects on 5 landraces phenology (R4, 150-153)</p> <p>Clear genetic differences between landraces have led to the provisional identification of more thermotolerant material which could be used for future breeding programmes and to select among existing landraces for increased tolerance to hostile environments.</p>
D4.2	Traits associated with drought, HSPs and cross tolerance to heat stress identified.

- P6 and P7 showed that maintenance of cell membrane stability, chlorophyll stability and thylakoid membrane stability were all confirmed to be important for heat stress tolerance.
- Indian partners proposed a high throughput screening using a Leaf Cell Membrane Thermostability (LCMT) approach to identify heat tolerance material.
- Clear genetic variation among landraces for LCMT was identified in a number of experiments both in African and India.
- There was a significant positive correlation between temperature and seed emergence.
- P3 identified a clear physiological effect of heat stress to be a reinforcement of photoperiodic requirement, with semi-insensitive material shown to become more sensitive at high temperatures.
- P1 and other partners demonstrated that high temperature also leads to decreased pod-set and increased vegetative development in a genotype-dependent way. Whether this is a direct effect of reduced fertility at higher temperatures leading to allocation of resources to vegetative growth or whether it is an intrinsic effect of temperature is unclear.
- The latter two results suggest that there is likely to be a direct interaction between the strength of photoperiod requirement and temperature in bambara groundnut, which is an important finding to consider in the development of photoperiod 'insensitive' material for non-equatorial regions.

D4.3	<p>Completed</p> <p>P6 used AFLP to try to distinguish landraces with greater cell membrane thermostability (R3, p206-216) A clear link between SLA and heat stress has been established and could potentially lead to a surrogate selection criterion. QTL for SLA were detected within the 'wide' population (R5, p39-45), so candidate genes could be tested through an analysis of genetic location. This could be coupled with the previous MPSS data from P4 (R3, p133-151) and the current leaf transcriptome (R5, p46-49) from P1 to try to identify candidate genes.</p>
D4.3	<p>Identification of traits associated with heat stress and identification of QTL for heat tolerance allowing the production of crosses contrasting for HS tolerance.</p>
D4.2	<p>Completed</p> <p>A root block experiment by P6 using the 6 landraces measuring the effect of heat-stress on <i>Fv/Fm</i> and other PS traits, including leaf membrane thermostability in irrigated and unirrigated plants revealed landrace differences (R1, p141-144) P6 evaluated four landraces for effects of heat stress (R2, 166-171) P6 measured cell membrane thermostability to identify tolerant and more susceptible material, for potential parents (R3, p202 – 205)) P4 reports use of MPSS data to generate a 132 gene oligoarray to evaluate gene expression change in leaf during drought stress (R3, p133-151). P2 planted 6 landraces, irrigated/non over 3 months; photochemical efficiency and yield (R4, p34-43) A detailed assessment of the effects of high temperature stress on the components of both leaf membranes and chloroplast function has provided a number of potential mechanisms, by which heat stress tolerance could be enhanced. The development of the recent leaf transcriptome should lead to the identification of candidate genes for further evaluation.</p>

WP5 Cold Stress

D5.1	Identification of traits associated with plant development at low temperatures in contrasting environments	
D5.1	<p>Partially Completed</p> <p>P1 used the TCRU to evaluate the effects of low and high temperature on two landraces for photosynthetic, phenological and yield traits (R1, p17-53)</p> <p>Development of BAMGRO-Stress by P1 (R2, p39-48)</p> <p>P1 grew 2 landraces in the TCRU to evaluate the effects of high and low temperature (R3, p32-50)</p> <p>BAMGRO-stress basic modules developed by P1 (R3, p51-57) P5 evaluation of effects of low night temperatures (R3, p152-163) P8, 6 landraces field sown over 4 months with and without irrigation (R3, 251-284) P9 sowing 4 landraces as night temperatures dropped found pod-set inhibited (R3, p295-296)</p> <p>P9 sowing 4 landraces as temperatures dropped (R4, 144-150)</p> <p>P1 carried out a coupled molecular/CE experimental analysis of the effects of cold stress on two landraces (R5, p51-72)</p> <p>A series of in-field experiments using either altitude or a range of sowing dates have examined the effects of low temperature on bambara groundnut development and yield. CE experiments have also examined the effects of a progressive reduction of temperature on both shoot and root development, with direct coupling to molecular experiments. The latter experiment is yet to be fully analysed (all data production is complete) but there are clearly differential effects between landraces for both shoot and root.</p> <p>Note: this aspect was originally assigned to P10. Non-completion of deliverables and non-production of reports led to P10 being removed from the project. Partial funding was transferred to P1 to attempt to continue this work as well as possible, but analysis of the coupled molecular/physiology experiment is not complete yet.</p>	
D5.2	QTL's associated with low temperature tolerance	
D5.2	<p>In progress/alternative approach</p> <p>Confirmed parents contrasting for low temperature tolerance have been crossed and are awaiting assessment. The two crosses currently available ('wide' and 'narrow') are not expected to contrast significantly for LTT.</p> <p>Due to this, we have developed a leaf transcriptome experiment from a temperature series and comparison across species for known genes to cold stress in other legumes. These candidates can also be mapped within bambara in subsequent experiments examining low temperature stress.</p>	
D5.3	Knowledge of independent and combined effects of temperature and photoperiod on plant development	

D5.3	<p>Partially completed</p> <p>P2 established protocols and began to evaluate the coupled effects of photoperiod and temperature through using 4 sowing dates covering a 10 week period (R1, p56-63) P10 analysed a 6 landrace experiment looking at the combined effects of low temperature and photoperiod (R2, p230-234) P1 carried out a coupled molecular/CE experimental analysis of the effects of cold stress on two landraces (R5, p51-72)</p> <p>Field experiments using photoperiod sensitive and insensitive material have been carried out at different average temperatures. This has been followed up by CE work. A clear effect of high temperature on reinforcing photoperiod requirement was identified by P3, but the effects of low temperature were not explicitly tested.</p>
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-In response to a series of sowing date experiments by P1 and other partners, it is clear that PSII efficiency declines with temperature.

-Seed emergence decreased significantly with cold temperatures, although genetic variation between landraces does exist.

-A detail CE column experiment at P1 demonstrated the effects of plant development and phenology at low temperature (28°C vs 23°C vs 18°C) in two landraces.

-Landrace-specific effects were seen for both root and shoot effects.

-Molecular analysis of this experiment generated the first comprehensive bambara groundnut leaf transcriptome and allows a detailed analysis of the 2,000 – 3,000 genes which show temperature and/or landrace specific changes.

-Crosses between two landraces shown to exhibit differential responses to low temperatures have been made, but have yet to be analysed.

- A clear reinforcement of photoperiod requirement by high temperatures was observed by P3 in CE work, which suggests lower temperatures reduce the stringency of photoperiod requirement, although this has not been explicitly tested.

AT6 Photoperiod Response

D6.1	<p>Response of photoperiodism on crop growth and phenology</p>
D6.1	<p>Completed</p> <p>P6 planted 10 landraces in 3 locations over 6 months at 15 day intervals to assess photoperiod effects. Results indicate significant effects of photoperiod on phenology, with maturity varying from 98 to 140 days, although landrace also influenced this (R1, p144-147)</p> <p>P3 evaluated the effects of photoperiod (10-14h) on three landraces in CE rooms (R2, p67 – p85) P6 evaluated effects of photoperiod through planting six or more landraces across a total of 6 months (R2, p172 – 179) P6 used a series of plantings to test whether there was a photoperiod effect in Bangalore (R2, p194-198)</p> <p>P2 assessed the effects of irrigated/non-irrigated, temperature and photoperiod through different sowing dates (R3, p93-100) P3 pot experiments show restricted root space will eventually restrict canopy growth and prevent pod formation; photoperiod may act by raising ABA levels (R3, p115-123) P6 sowing date experiment to examine photoperiod effects (R3, 217-224)</p> <p>P6 multiple planting dates and yield component analysis (R4, p111-123) P3 screened multiple lines and already identified lines to detect/confirm photoperiod insensitivity (R3, p61-p64). P9 planting of 5 landraces confirms relationship between RWC and SLA (R4, p153-158)</p> <p>Extensive work has been carried out in field and in CE rooms to evaluate the effects of photoperiod requirement on crop growth and phenology</p>

-Six CE room experiments have tested 250 lines for photoperiod requirement, identifying a number of landraces showing different levels of photoperiod sensitivity, including the least sensitive TZA-1505.

-Effects of increasing temperature on these lines suggested that higher temperatures reinforced photoperiod sensitivity.

-In field experiments in Ghana five moderately insensitive landraces were identified and their interaction with temperature evaluated.

-Pot-bound plants (presumably with higher ABA levels) negatively affected both flowering and pod filling.

-Tiga Necaru, also reported to be photoperiod insensitive (although less so than TZA-1505 as shown in direct comparisons) is one parent of a controlled cross and is ready to be evaluated further. Material from this cross is currently planted in three photoperiod regimes (12/14/16hr) in the new FutureCrop tropical glasshouse facility at P1.

- Both planting date experiments and CE experiment show that while there can be a photoperiod effect on flowering, it is the photoperiod effect on podding which has the strongest influence.

-Controlled crosses between the insensitive and sensitive material, to introduce (partial?) photoperiod insensitivity into bambara groundnut lines is underway

D6.2	Tapping the genetic potential for photoperiodic response and low temperature tolerance.
D6.2	<p>Completed</p> <p>P3 screened 105 landraces for evidence of photoperiod insensitivity. Four potential lines were identified (R2, p86-87)</p> <p>P3 confirms that TZA-1505 is insensitive at lower temperature, but sensitive to pod fill at higher temperatures; Tiga Necaru is confirmed insensitive at 14h, but not 16hr (R3, p122-124)</p> <p>P3 confirms photoperiod neutral lines and screens 146 more accessions (R4, p61-68)</p> <p>A clear interaction between photoperiod insensitivity and temperature, with high temperatures reinforcing photoperiod sensitivity was identified, along with material for future breeding</p>
D6.3	<p>Knowledge of independent and combined effects of photoperiod and temperature on pod initiation and duration</p>
D6.3	<p>Completed</p> <p>P2 established protocols and began to evaluate the coupled effects of photoperiod and temperature through using 4 sowing dates covering a 10 week period (R1, p56-63)</p> <p>P10 analysed a 6 landrace experiment looking at the combined effects of low temperature and photoperiod (R2, p230-234)</p> <p>P3 has carried out experiments in controlled environments to evaluate this interaction (R2, p86-87; R3, p122-124; R4, 61-68)</p> <p>The interaction between photoperiod requirement and temperature has been determined and strongly insensitive material identified. Agroecological mapping suggests that a number of regions would be able to grow bambara groundnut productively, if suitable photoperiod insensitive material could be obtained. Insensitivity could also solve the potential problem with discordance between rains and sowing dates, which has been suggested to be one cause of erratic yields in bambara groundnut in some countries.</p> <p><i>We are currently analysing the photoperiod insensitive material identified by P3 at P1, with a photoperiod series (12 hours/ 14 hours / 16 hours) in the new Tropical glasshouses (FutureCrop) at Nottingham, UK. The analysis is coupled with speculative crosses to try to introduce photoperiod insensitivity into a wide range of African and Indonesian germplasm.</i></p>

AT3 – Nutritional/Functional Evaluation – WP7

D7.1	Food consumption patterns, existing preparation methods and limitations of food uses of bambara groundnut identified and documented for Africa and India	
D7.1	Completed Seed physical and adsorption characters determined for 5 local landraces by P5 (R3, p165-170) Survey results for Africa are reported in WP9 and extensive baseline data have been gathered and analysed	
D7.2	Antinutritional factors in bambara groundnut identified and appropriate inactivation processes developed	
D7.2	Completed Determination of antinutritional tannins in 2 landraces by P5 (R3, p171-172) P6 determined phenols in seed and sugars in leaf, +/- Bradyrhizobium (R4, p124-129)	
D7.3	Effects of abiotic stresses viz. drought, heat, cold and photoperiodic control of phenology on physico-chemical and antinutritional content of selected bambara groundnut genotypes quantified	
D7.3	Completed P6 evaluated 95 nodulation strains of (Brady)rhizobium for nod and nif genes by PCR and tested 2 identified strains for ability to nodulate on bambara groundnut (R2, p180-184) P5 Effects of drought stress on seed composition determined for 5 landraces (R3, p172-174) Effects of (B)rhizobium on leaf sugar content and seed yield (R3, p225-230)	
D7.4	Traditional means of storage identified, improved and new appropriate storage methods established	
D7.4	Completed P5 evaluated current storage approaches (R2, p126-131) and proposed improvements	
D7.5	Physico-chemical and nutritional properties of bambara groundnut determined	
D7.5	Completed P5 developed methods for protein extraction and examining functional properties of bambara groundnut seed (R1, p82-84) P6 studied 7 landraces for leaf sugar/phenolics (R1, p148-149) P2 (SC3) evaluated water absorption characteristic of 5 landraces in relation to cooking (R2, p49-56) P5 evaluated the proximate composition of 5 local landraces (R2, p132-134) P8 carried out proximate composition analysis of 7 local landraces (R2, p215, 216) P5 (SC4) proximate analysis on 6 local landraces (R3, p103-106). P8 proximate analysis of 6 landraces (R3, p285) P8 nutritional analysis of 6 landraces (R4, p137-141) P5 has partially completed a digestibility analysis of landrace leaves for ruminants (R5, p78-84)	
D7.6	Recipes and processed products developed for diversified uses in Africa and India	
D7.6	completed P5 developed methodologies for bambara sauce and the development of bambara groundnut milk (R1, p85-86) P5 High Quality Bambara Flour (HQBF) substituted in a number of dishes and taste panel valuation, with many HQBF favoured (R4, p81-90) P5 developed and analysed bambara groundnut milk, generally preferred to soyabean (R4, p90-93)	

- Five partners and 1 subcontractor have produced detailed information on proximate analysis, anti-nutritionals, such as phenols and trypsin-inhibitors and the effects of different stresses on nutritional composition.
- The effects of different *Rhizobium* strains suitable for India on nodulation and yield have been extensively investigated.
- Extensive questionnaires for African partners have revealed the preferences of farmers, merchants and processors.
- Current storage methods in Ghana have been surveyed and improvements developed. A number of products (including flours and milk) have been surveyed to indicate preferences, with bambara groundnut comparing favourably to other alternatives, such as soya.
- A reasonably simple processing procedure for bambara groundnut to give High Quality Bambara Flour (HQBF) was found to reduce poor flavour and antinutritional content.
- Pastry products made by partial substitution of wheat with HQBF were positively received by a taste panel, as was milk made from HQBF.
- Composition of seed was found to be a useful supplement to the diet and the nutritional quality of non-seed parts of the plant were found to be good for fodder.
- A method has been developed for 'bamba sauce' as a potential value added product.

WP8 GxE Interactions

D8.1	Potential variation in agronomic traits determined	
D8.2	Traits with high heritability and genotypes with high stability identified	
	Completed Development of BAMGRO-Stress by P1(R2, p39-48) P1 describe module development for BAMGRO-stress (R3, p50-56). Equation development and testing of BAMGRO-Stress by P1 (R4, 26-32) and subsequent implementation into the AQUACROP suite of FAO models. Substantial genetic variation has been detected for most traits evaluated	
	Partially completed P3 With the recent death of Prof Joergen Christiansen, the integration of the data originally envisaged cannot be completed in the immediate future. Soren Joergensen (recently awarded a PhD while working on BAMLLINK) will be involved, but this will take time (R5, p73-77) A detailed stability analysis has been conducted using a range of African and Indonesian landraces by a PhD student at P1 and reported to the recent conference (R5, p161-171)	
D8.3	Growth and yield potential of bambara groundnut genotypes identified for contrasting agro-ecological zones and soil conditions.	Partially completed As for 8.2 In addition, the GRASP database as been initiated at UoN (http://grasp.nottingham.ac.uk/) which will hold geospatially-anchored crops data, with 'genotype' as the basic descriptor, with associated genetic, physiological, nutritional and end-user data. Once fully developed, this will hold the bambara groundnut data generated in this project and allow agro-ecological matching searches, with a longer term aim to incorporate climate change scenario data. Selected slides from a recent presentation are given in Appendix I.

- Five landraces suitable for growing in western India have been identified and planting times determined. Seed are being multiplied.
- In Gujarat (P6), detailed leaf anatomy traits were correlated to drought resistance and strong genetic determination of a number of traits found.
- The Global release though the FAO of the bambara groundnut version of the AQUACROP model as a development of the BAMGRO-stress model developed here makes bambara groundnut the only underutilised species to have been implemented in the AQUACROP suite of models.
- BAMGRO-stress was developed to include landrace specific parameters and provides one platform for the eventual integration of high level crop models and the underlying molecular and physiological mechanisms determining the trait
- A full BBSRC UK proposal is currently under consideration to link mathematical modelling of canopy development in bambara groundnut with the high level BAMGRO and AQUACROP models.
- GRASP is being developed as an integrated Geospatially-anchored database which will allow agro-ecological matching and climate change scenarios to be run on a range of crops and pests, including bambara groundnut.

AT4 –End-user benchmarking – WP9 Africa

D9.1	Ideotypes for African bambara groundnut genotypes defined
D9.1	Completed From survey work, farmer's desired traits have been identified and from the genetic work, we have now identified a number of potential loci which influence those traits and could be used for selective breeding.
D9.2	Criteria for matching preferences of local growers and end-users in African partners
D9.2	Completed P5 established a base-line for bambara groundnut using an extensive structured survey in two contrasting regions of Ghana (Transition and Guinea Savannah) with a total of 190 producers and 290 processors (R1, p87 – 136) P10 reported a structured survey on 184 farmers evaluating the factors farmers perceive as constraints for bambara groundnut (R1, p180-193) P2 surveyed 10 districts in Botswana and 3 groups (growers, consumers, traders) (R2, p57-65) P10 surveyed 59 farmers on cultural practice and current constraints (R2, 235-243) P2 surveyed government institutions in Botswana (R3, p106-107) P8 survey of 30 farmers in Namibia (R3, p285-286) P8 structured survey of 75 farmers (R4, p141-143)
D9.3	Factors limiting utilization of bambara groundnut in African partners identified
D9.3	Completed P2 determined a seed pre-soaking protocol to encourage germination (R3, p108-112). Nutritional, storage and end-product factors have been investigated (see WP7)
D9.4	Acceptability criteria for consumer demand and market potential of bambara groundnut in African partners defined
D9.4	Completed Acceptability criteria for consumer demand and market potential of bambara groundnut in African partners have been defined by Partners and will form basis of ideotype breeding, currently initiated at P1, P1, P5 and an Indonesian partner are currently pursuing funding for an international breeding programme (BAMBREED) to implement the identified requirements.

- Detailed ideotypes desired by farmers have been defined.
- Perceptions of and reasons for growing bambara groundnut determined in four African countries.

AT4 – End-user benchmarking – India

D10.1	Development of Bambara groundnut as a potential new crop in the arid-zones in Gujarat
D10.1	Completed P6 established a benchmarking exercise and assessment of the suitability of bambara groundnut for India and identified regions and farmer for growing/demonstration trials (RI, p150-152) while trying to multiply material. Extensive research has been carried out in India on this crop and the best landraces are being pursued for more extensive introduction.
D10.2	Criteria for matching preferences of local growers and end-users in Indian partner regions
D10.2	Partially completed As bambara groundnut was not pre-existent in India, this benchmarking exercise only yielded fairly general considerations, although farmers preferences were noted and farmers were introduced to the crop.
D10.3	Factors limiting potential utilization of bambara groundnut in Indian partners identified
D10.3	Not complete Seed multiplication has been the major limitation on assessing D10.3
D10.4	Acceptability criteria for consumer demand and market potential of bambara groundnut in Indian partners defined
D10.4	Not complete As for D10.3. The crop clearly has potential and farmers were interested in the crop. Indian partners and the Indian government, through the Groundnut Research Centre, continue to work on this introduction.

Limited seed availability has made widespread testing of consumer acceptance not possible to carryout

- Farmers have been introduced to the crop in demonstration plots

SECTION 2 Maximising international impact of BAMLINK

2. AQUACROP/BAMGRO

OVERVIEW of BAMGRO/AQUACROP MODEL

INTRODUCTION

The agricultural systems in semi-arid regions of sub-Saharan Africa are normally characterised by extreme climatic conditions. Some of the most resilient crop species such as sorghum (*Sorghum bicolor*, L. Moench), pearl millet (*Pennisetum americanum*) and cowpea (*Vigna unguiculata*) have originated from this region. Globalization challenges the existence of these indigenous crops with less resilient, but more popular crops like maize (*Zea mays*) and bean (*Vicia faba*), which are higher yielding under favourable climates. In addition, the improved breeding programmes of major crops have widened the gap between the cosmopolitan and traditional crops. Bambara groundnut (*Vigna subterranea* (L.) Verdc) is one such indigenous legume with significance as a source of protein in sub-Saharan Africa. Although there are many growth simulation models for a range of major crops, there have been few attempts to model underutilised species for which factors controlling growth and development are not well understood. A crop simulation model for an agronomically and nutritionally variable crop like bambara groundnut will provide the framework for scientific cooperation to rapidly integrate new knowledge and prioritise future research on an under-researched and underutilised species. This new approach to model bambara groundnut responses to major abiotic stress factors provides a platform for easily incorporating other biotic and abiotic factors and extending the model to more landraces and ultimately varieties of the crop. These modelling approaches linked with integrated research have useful lessons for the modelling of other underutilised crops and their potential for future agricultural systems.

BAMGRO Model structure and overview

BAMGRO consists of different sub-modules that deal specifically with weather, crop growth, soil water, temperature and photoperiod. Fig. 1(a) shows the interactions of the main components within the model. The weather module calculates the thermal time for the developmental processes of the crop using weather data and cardinal temperatures. The crop growth module simulates canopy development (*LAI*), dry matter production (*LLG*) by means of radiation interception and yield through the partitioning coefficient (Fig. 1(b)). The soil water sub module calculates root growth, root water uptake, water limited growth (*WLG*) and soil water balance: as inputs through rainfall and irrigation and various means of water losses through the system (Fig. 1(c)). The temperature module calculates the temperature stress index and the photoperiod module estimates the day length factor considering the available day length and critical photoperiod 12 h. BAMGRO is a process-oriented model that simulates a crop carbon balance and a soil water balance. The carbon balance includes daily inputs from photosynthesis and conversion of carbon into crop tissues, and losses due to abscised parts. The simulation of growth includes leaf addition, senescence, leaf area expansion, pod addition and pod filling. The main time step in BAMGRO is 1 day, but thermal time is calculated hourly and integrates over the time course of day. The model uses a daily input of weather data, and is designed to simulate canopy development, crop biomass (growth), dry matter partitioning within the crop, yield and soil water uptake. The

The main inputs are daily weather data: as minimum temperature, maximum temperature, solar radiation, saturation deficit (SD) and rainfall. The major soil characteristics are soil bulk density and soil depth. These files were created from glasshouse experiments at Tropical Crops Research Unit (TCRU), University of Nottingham, UK and from field experiments in Africa (Table 1). Landrace

Input Data Files

Model description
BAMGRO model is developed with input data files and various sub modules as explained below.

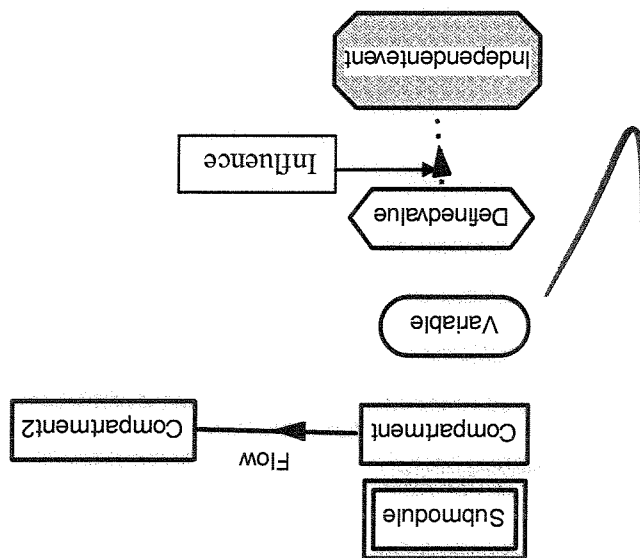
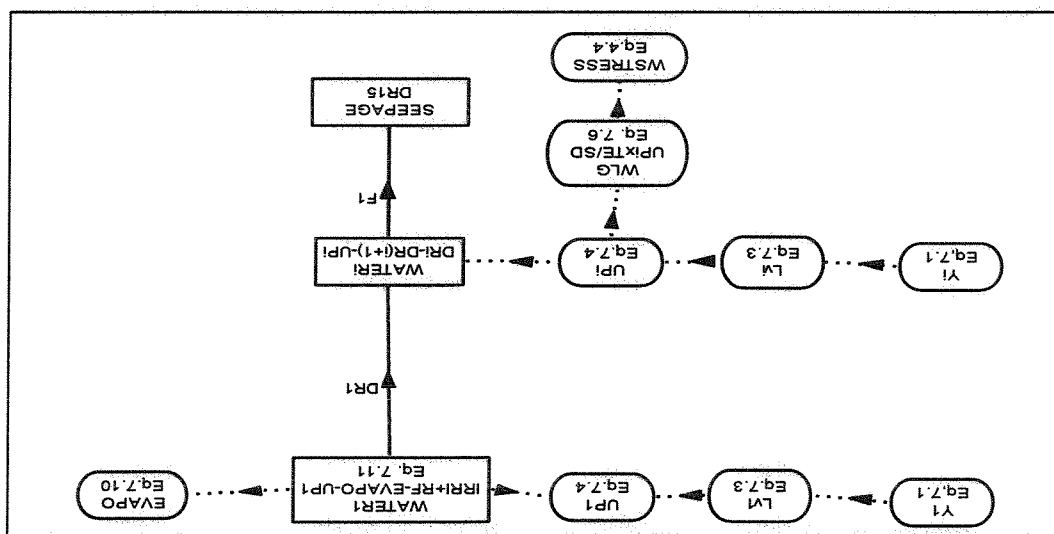


Fig. 1. Schematic overview of data flow through the sub-modules in BAMGRO model: (a) interactions of sub-modules and input files (b) detailed diagrammatic representation of crop growth module (c) detailed diagrammatic representation of soil water module.

(c) Soil water module



(b) Crop growth module

coefficients are the second main input file to BAMGRO. They were derived from various glasshouse (TCRU-Nottingham) and field experiments in Africa.

Table 1. Summary of experiments used for model data sets.

Experiment	Data sets	Location and year
Growth room	Calibration	University of Copenhagen, Denmark (2006-2008)
Glass house	Calibration	University of Nottingham, UK (2006)
Glass house	Validation	University of Nottingham, UK (2007)
Glass house	Validation	University of Nottingham, UK (2008)
Field	Validation	Botswana (2006-2008)
Field	Validation	Swaziland (2002-2003)
Previous	Calibration	University of Nottingham, UK

Weather Module

The main role of the weather module is to read daily weather data from input files that were created using experimental measurements described in Table 1. It reads daily weather parameters (maximum and minimum air temperature, solar radiation, SD , rainfall in field sites and irrigation under controlled environmental experiments) from the weather files. In addition to the above parameters, relative humidity and wind speed are read from the weather file when available.

Thermal time is calculated hourly and integrated over the day (24 hours). This daily thermal time is used in the crop growth module to determine the accumulated phenochrons thereby the stage of growth. The accumulation of thermal time over the growing period is calculated according to Eq. 3 as applied in BAMFOOD project model.

$$T_{mean} = \frac{(T_{max} + T_{min})}{2}$$

1

Hourly thermal time is accumulated over 24 h period using hourly temperature values $T_{d(i)}$

$$T_d = T_{mean} + 0.5 \times abs(T_{max} - T_{min}) \times \cos(2.618 \times (-14))$$

2

$$\frac{dT}{dt} = Min \left[\left(\frac{T_{d(i)} - T_{base}}{24} \right), 0 \right]$$

For $i = 1, 24$

3

Where,

T_{mean} = daily mean temperature ($^{\circ}C$)
 T_{min} = daily minimum temperature ($^{\circ}C$)
 T_{max} = daily maximum temperature ($^{\circ}C$)
 T_{base} = base temperature ($^{\circ}C$)
 T_{opt} = optimum temperature ($^{\circ}C$)

$T^{(a)}$ = hourly temperature in hour t ($^{\circ}\text{C}$)
 TT = cumulative thermal time (degree days)

Crop Growth Module

The accumulated thermal units produce the first leaf for the first instance and thereafter that rate of new leaf production is dependent on daily accumulation of thermal time until maturity. Leaf area is produced as a function of leaf number. Daily plant growth is computed by converting daily intercepted Photosynthetically Active Radiation (PAR) into plant dry matter using a crop-specific radiation use efficiency parameter. The daily growth through light interception is computed as a function of LAI , radiation use efficiency (ϵ_s) and light extinction coefficient (k). The amount of new dry matter available for growth in each day is modified by the most limiting of soil moisture or solar radiation. Above ground biomass demands the major part of the carbohydrates produced each day and at the end of the day carbohydrates not used for above ground parts are allocated to roots, subjects to certain minimum requirements.

The pod number is inversely proportional to the leaf number with accumulation of thermal time; thereby a control is implicitly operated within the model to regulate pod formation. Once pod filling has started, the model computes the growth of pods based on user defined maximum rate. If the daily available photosynthates are insufficient to achieve the potential growth rate of pods, a fraction of carbohydrates can be remobilized from vegetative parts and roots to reproductive sinks each day based on demand of the reproductive organs. Pods are allowed to grow until physiological maturity provided sufficient resources for plant growth are available. If the growth resources are inadequate, growth is terminated prior to physiological maturity.

Soil Water Module

The BAMGRO-soil water module simulates root growth, root distribution, root water uptake and soil water balance from sowing until maturity for different bambara groundnut landraces. The soil is represented as a one dimensional profile; it is homogeneous horizontally and consists of number of soil layers. The total soil depth is assumed to be 1.5 m. This profile is divided into 15 soil layers each of 10 cm depth.

Water stress index

For the purpose of the model it is assumed that growth of the unstressed crop is limited by the solar radiation available for photosynthesis process and its photosynthetic capacity. This is defined as light limited growth (LLG). When the growth is reduced by water limitation this is termed as water limited growth (WLG). If the crop is exposed to a restriction of water supply and the water uptake by roots is insufficient to replenish transpiration at potential growth rates the plant is exposed to moisture stress. The BAMGRO model contains a number of relationships in which the growth and developmental performances of the crop are modified by the water stress that is experienced. To model the effect of soil moisture stress on growth and development, water balance and a relation between crop growth and water availability is considered. The value is given from zero to one, representing maximum stress and no stress respectively. The basis of calculation of water stress index was derived from BAMnut and modified for present model BAMGRO.

$$WSTRESS = \left(\min \left(\frac{WLG}{LLG}, 1 \right) \right)$$

Where,

$WSTRESS$ = water stress index
 WLG = water limited growth
 LTG = light limited growth
 T_{high} = ceiling temperature ($^{\circ}C$)

Temperature Module

The temperature sub module considers the output from the weather module as input to calculate temperature stress index according to Eqs. 5 and 6 depending on the mean temperature. When the crop is exposed to a range of temperature within the boundary line of base (T_{base}) and ceiling (T_{high}) temperatures, it results in temperature stress effects on growth and development. However temperature stress can be further divided into heat and cold stress based on the mean temperature and agro-ecological adaptation of the landrace. Similar to $WSTRESS$ the value of temperature stress index ranges between zero (maximum stress) and one (no stress) (Fig. 2).

$$TSTRESS = \frac{T_{mean} - T_{base}}{T_{high} - T_{base}} \quad \text{for } T_{mean} \leq T_{lower}$$

$$TSTRESS = \frac{T_{high} - T_{upper}}{T_{high} - T_{lower}} \quad \text{for } T_{mean} > T_{upper}$$

Where,

$TSTRESS$ = temperature stress index.
 T_{high} = ceiling temperature ($^{\circ}C$)
 T_{lower} = lower threshold level of temperature ($^{\circ}C$)
 T_{upper} = upper threshold level of temperature ($^{\circ}C$)

BAMGRO calculates temperature stress index according to Eqs. 5 and 6, and two threshold levels are set as lower (T_{lower}) and upper (T_{upper}) for each landrace as a novel approach in present study. When T_{mean} is lower than the T_{lower} crop experience a cold stress while temperatures above T_{upper} it causes a heat stress (Fig. 2). Based on the experimental evidences in glasshouse experiment (TCRU-2006) the lower and upper threshold levels for Swaziland landrace, Uniswa Red are set as $17^{\circ}C$ and $35^{\circ}C$ respectively. However, the Namibian landrace, S19-3 reported a cold stress with LT ($23 \pm 5^{\circ}C$) while no significant heat stress with HT ($33 \pm 5^{\circ}C$) and therefore, BAMGRO uses $24^{\circ}C$ and $38^{\circ}C$ for lower and upper threshold levels.

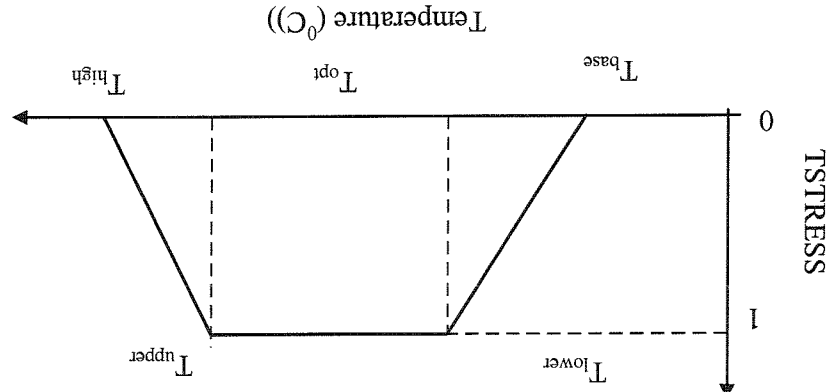


Fig. 2. Diagrammatic representation of variation in temperature stress index ($TSTRESS$).

Photoperiod Module
Bambara groundnut is a short day crop and pod formation is regulated by photoperiod. The experimental evidence from growth room experiments at the University of Copenhagen, Denmark, showed that photoperiod is positively correlated with rate of leaf production. Therefore BAMGRO calculates the day length factor to adjust the daily rate of change in new leaf production when the crop is grown above 12 h day length. The gradient of the linear function of rate of leaf production with different day lengths is simply considered as the day length factor (DL_{fac}) (Eqs. 7, 8) as a new approach in BAMGRO model.

$$DL_{fac} = 1 \quad \text{for } DL \leq 12 \text{ h} \quad 7$$

$$DL_{fac} = p_1 \times DL \quad \text{for } DL > 12 \text{ h} \quad 8$$

Where,

$$\begin{aligned} DL &= \text{day length (h)} \\ DL_{fac} &= \text{day length factor} \\ p_1 &= \text{landrace parameter} \end{aligned}$$

BAMGRO response to abiotic stress

Environmental stresses represent the most limiting factors for agricultural productivity. Apart from the biotic stress caused by plant pathogens, there are number of abiotic stresses such as extremes in temperature, drought, salinity, heavy metals and radiation which all have detrimental effects on plant growth and yield. However certain plant species and ecotypes have developed various mechanisms to adapt such stress conditions. According to the evidences from TCRU-experiments, bambara groundnut reported that growth, development and yield are impaired by abiotic stress factors. The present study considers soil moisture and temperature as major abiotic stress factors that influence growth and yield of bambara groundnut. Depending on the timing, severity, duration and landrace the type of stress varies for different plant processes. BAMGRO model distinguishes three stress effects due to independent and cumulative effects of drought ($WSTRSS$) and temperature stress ($TSRSTS$) as: on leaf production, leaf senescence and dry matter partitioning. These stress indices are modifiers of the target model parameters and varies in value from one when the effect is non-existent, to zero when the effect is maximum.

Modelling software: Model Maker 3.0

BAMGRO model is formulated and run using the Model Maker software (Version 3.0). This uses a simple drag and drop approach to simulation modelling and is more intuitive and added extra features to improve optimisation and analysis. The software provides facilities to formulate the model using differential functions, conditional applications, time triggers events and run the model on user defined time steps. The coding of BAMGRO in Model Maker is presented in Appendix 1 (Model Maker manual).

Efficiency criteria

In order to assess model performances and provide an objective evaluation of the "closeness" of simulated (S) vs. measured (M) values, a number of indicators are used. There are different goodness-of-fit measures and they will each be sensitive to different aspects of model (mis. behaviour) (Wainwright and Mulligan, 2002). However the choice of an appropriate measure is important in robust model evaluation.

Visual Evaluation

This is used to evaluate in a subjective way model performance, especially related to systematic behaviour (under or over estimation).

Gradient (b) and Intercept (a) of The Linear Regression

This involves an analysis of the simulated (S_i) and measured (M_i) values.

$$S_i = a + b \cdot M_i$$

Simple t-test is performed to check the significant deviations of slope of the regression line (a) and the intercept (b) from the ideal line of identity (1:1) in which the slope is one and intercept is zero.

The Nash and Sutcliffe (N-S) (1970) Model Efficiency Measure

$$N-S = 1 - \frac{\sum_{i=1}^n (M_i - \bar{M})^2}{\sum_{i=1}^n (M_i - S_i)^2}$$

N-S is the measure of the mean square error to the observed variance. If the error is zero, then N-S=1, and the model represents a perfect fit. If the error and observed variance are equal, then N-S=0 and the observed mean value is as a good representation of the model. A negative N-S value indicates that the error about the model is greater than the error about the mean (very poor fitting model).

Mean Absolute Error (MAE)

This is the mean absolute deviation between the simulated (S_i) and measured (M_i)

values.

$$MAE = \frac{\sum_{i=1}^n |M_i - S_i|}{n}$$

Model validation results

Canopy development

The BAMGRO model is intended to predict the performance of different genotypes under variable climates. Therefore the individual model comparison for LAI from glasshouse experiments in Nottingham, field trials in Botswana (Notwane) and Swaziland (Malikerns and Luvu) were pooled for each landrace (Fig. 3). Overall, simulated LAI correlated well with measured values for the two tested landraces; S19-

3 (N-S, 0.84) and Uniswa Red (N-S, 0.80) with maximum MAE less than ± 0.50 . The intercept of the regression line was not significantly different ($p > 0.001$) to the intercept (zero) of 1:1 line and the simulations starts through the origin. However, the slope of regression line was significantly ($p < 0.001$) lower to the slope (one) of 1:1 line explaining the observed underestimation.

Total Dry Matter

Overall, simulated TDM correlated well with measured values for all tested landraces when it was compared with combined data sets from all the experiments, corresponding to each landrace. According to the results from statistical analysis, simulation of biomass production is well correlated with the line of identity (N-S) and MAE ranges from 48.8 to 100 g m^{-2} (Fig. 4). However, the slope of the regression line is significantly ($p < 0.05$) lower values (ranges from 0.81 ± 0.08 to 0.86 ± 0.14) compared to the slope (one) of 1:1 line indicating under estimation of the dry matter production. The intercept of the regression line (ranges from 0.22 ± 0.11 to 0.26 ± 0.11) was not significantly different ($p > 0.05$) to the intercept (zero) of 1:1 line explaining initiation of dry matter production simulations from the origin.

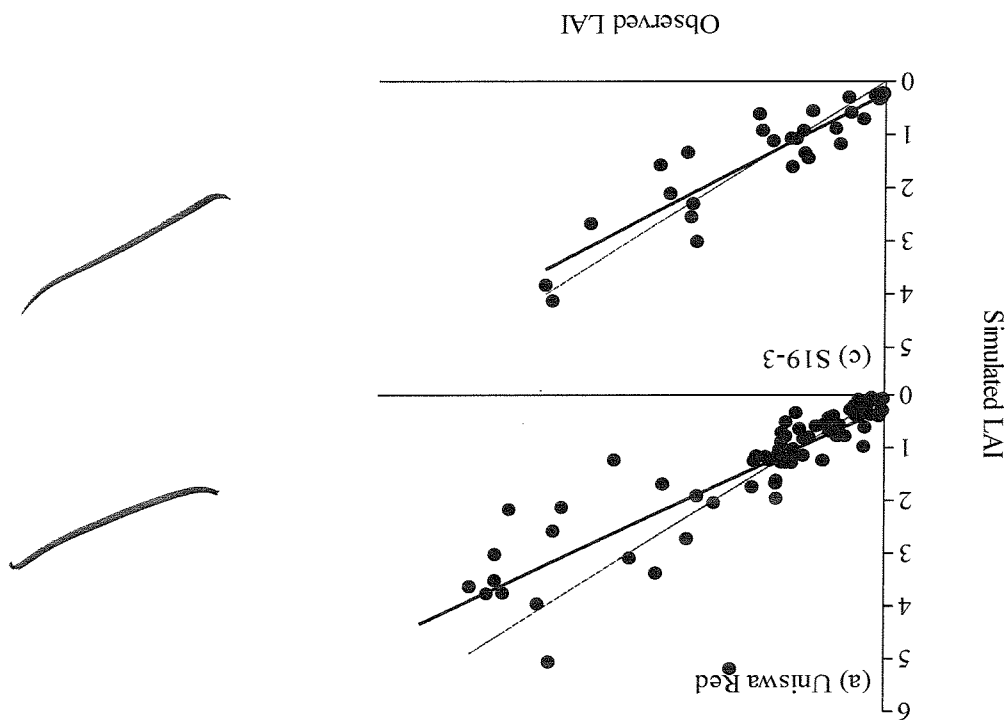
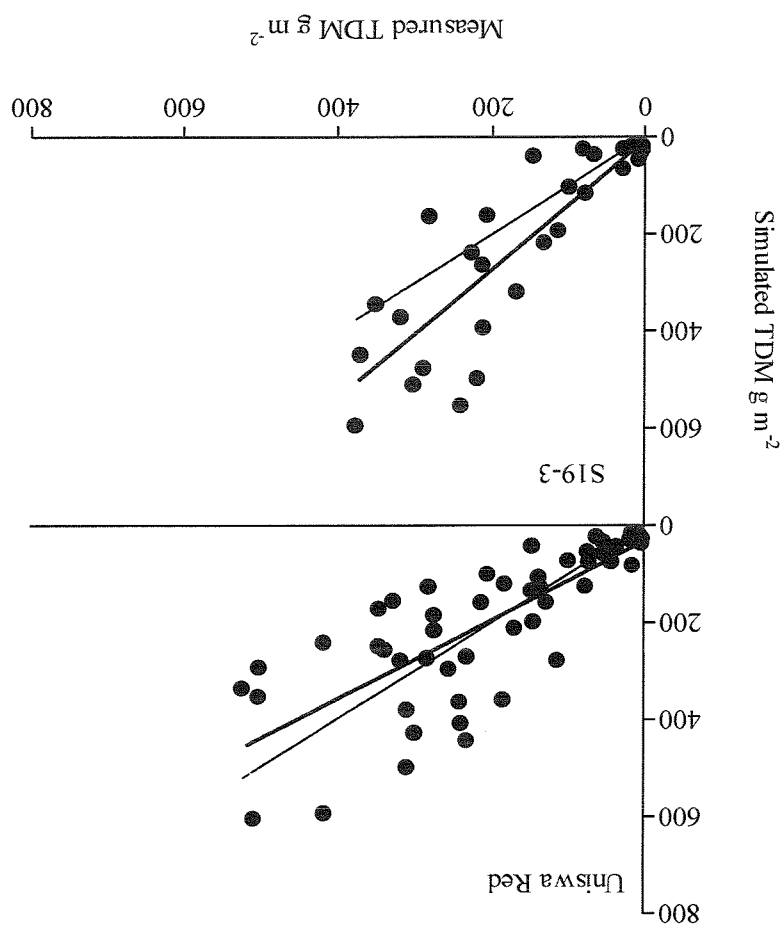


Fig. 3. Comparison between measured and simulated LAI for tested four landraces in glasshouse experiments, Nottingham, UK and field sites in Botswana and Swaziland (a) Uniswa Red (b) S19-3 1:1 line (---) and regression line (—).

Fig. 4. Comparison between measured and simulated TDM for tested landraces in glass house experiments, Nottingham, UK and field sites in Botswana and Swaziland (a) Uniswa Red (b) S19-3 1:1 line (---) and regression line (—).



Dry Matter Partitioning

Dry matter partition among the above ground parts as leaf, stem and pods was tested for independent data sets from Nottingham, UK (TCRU-2007 and TCRU-2008) and are shown in Fig 5 and 6 for Uniswa Red and S19-3 respectively. In general, simulation results of leaf weight that are calculated through F_{Leaf} were quite close to the observed values in Nottingham for both landraces (N-S ranges from 0.35 to 0.90). However stem weight was simulated with relatively lower N-S values (ranges from 0.20 to 0.90) compared to leaf weight simulations. The model validation results for individual pod weight by means of F_{Pod} are explained under yield formation.

Dry matter partitioning functions developed by means of changes over the vegetative and reproductive phases are successful in capturing the simulations of leaf weight and stem weight. Decrease in dry matter flow towards leaves and corresponding increase in stems over the vegetative phase and linear increase in fractional partition to pods by

switching to reproductive phase provides a strong framework for modelling dry matter partitioning in indeterminate crops.

Yield

Simulated pod yield correlated better with glass house measurements for S19-3 than Uniswa Red with higher correlation (N-S varies from 0.88 to 0.98) and MAE less than 25 g m^{-2} (± 16 to 21 g m^{-2}). Similar comparison of pod yield simulations was observed in Uniswa Red (N-S varies from 0.72 to 0.80) but HT in the 2008 season reported an overestimation especially towards the end of the season so that the N-S was poorly explained in this case. Pod yield varied with sowing dates and the model simulates the effect of variable climatic conditions in Botswana on yield formation (Fig. 5 and 6). BAMGRO was finally tested for two field trials in Swaziland (Malikerns and Luve). Yield showed a strong correlation with soil moisture limited condition which the model simulates successfully. The yield simulation for the drought affected Uniswa Red in Luve experiment was well correlated with measured values having N-S, 0.91 and lower MAE $\pm 9.4 \text{ g m}^{-2}$. However it was underestimated at Malikerns where the crop experienced non limiting moisture environment.

The model comparison for pooled data on yield over the season for each landrace correlated well with measured value. The statistical comparison of the model for the two tested landraces (Uniswa Red, S19-3), is well correlated with the line of identity (1:1 line) indicating acceptable correlation coefficient (N-S) for Uniswa Red (0.73) and S19-3 (0.87). The slope of the regression line in Uniswa Red (0.979 ± 0.066) and S19-3 (1.012 ± 0.061) and the intercept are not significantly different from slope of one the intercept (zero) of 1:1 line thus indicating the initiation of yield simulations through the origin.

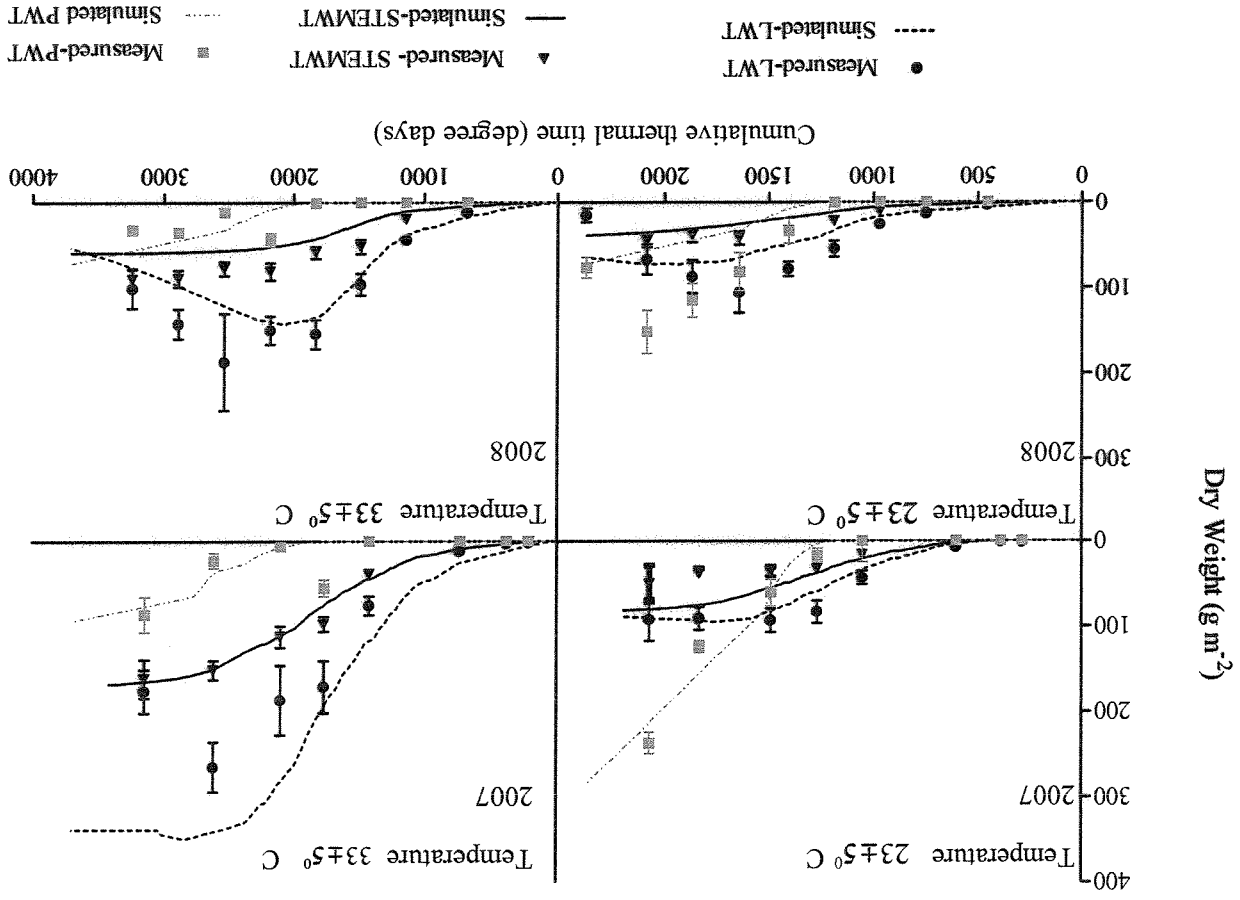


Fig. 5. Validation of leaf weight (g m^{-2}), stem weight (g m^{-2}) and pod weight (g m^{-2}) with cumulative thermal time grown under glass house conditions for Uniswa Red grown at low temperature ($23 \pm 5^\circ \text{C}$) and high temperature ($33 \pm 5^\circ \text{C}$) with drought at 77 (TCRU 2007 experiment) and 33 (TCRU-2008 experiment) respectively.

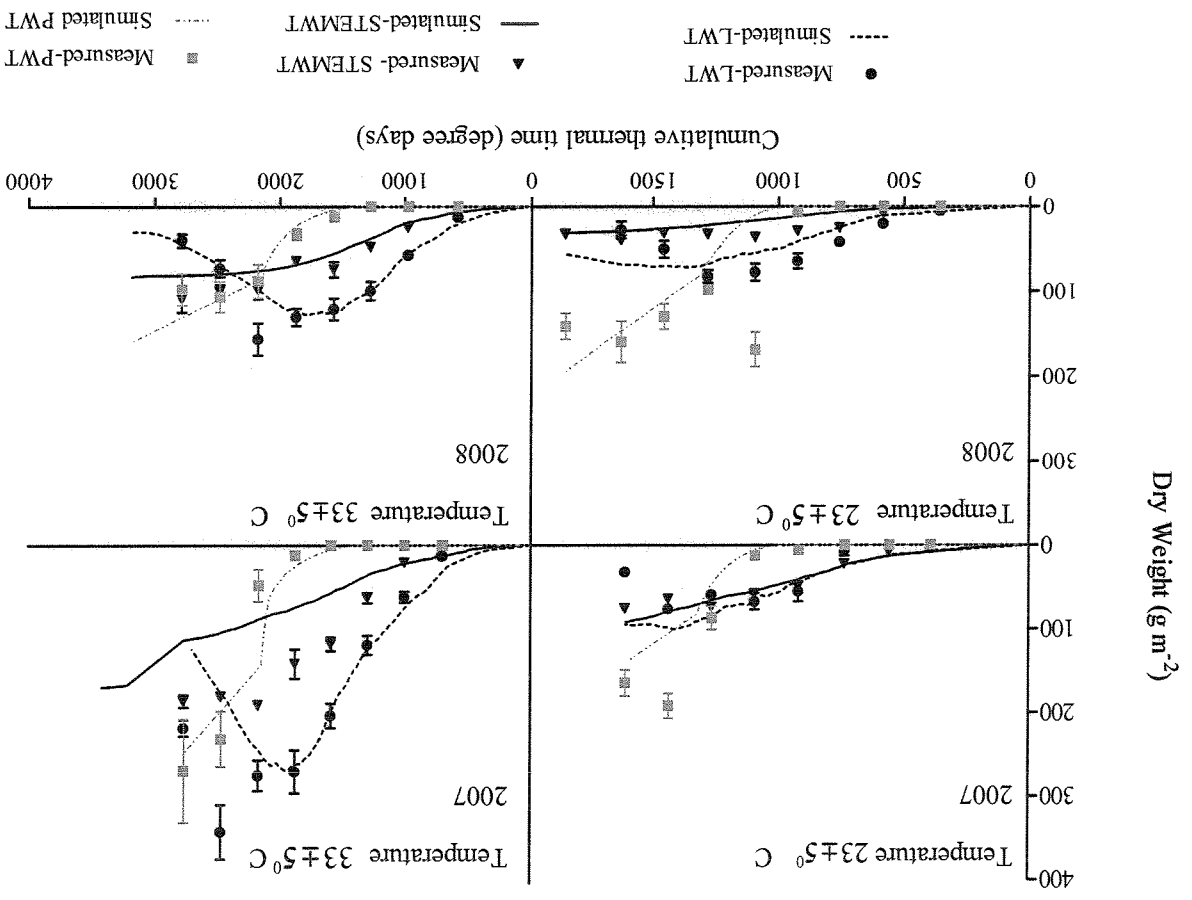


Fig. 6. Validation of leaf weight (g m^{-2}), stem weight (g m^{-2}) and pod weight (g m^{-2}) with cumulative thermal time grown under glass house conditions for S19-3 grown at low temperature ($23\pm 5^{\circ}\text{C}$) and high temperature ($33\pm 5^{\circ}\text{C}$) with drought at 77 (TCRU 2007 experiment) and 33 (TCRU-2008 experiment) respectively.

BAMBREED

an outline for an initial breeding programme for bambara groundnut.

Partners:

- 1: Nottingham campus (University of Nottingham, UK)
- 2: Semeniyih campus (University of Nottingham in Malaysia)
- 3: Indonesia National bambara groundnut breeding programme, Gresik and Bogor
- 4: Ghana, Crop Research Institute, Kumasi.
- 5: International - Crops for the Future
- 6: International - Crops for the Future Research Centre.

Outline

To be able to take the information generated during the past 18 years of bambara groundnut research co-ordinated at Nottingham and to begin to make a difference on (literally 'in') the ground it is essential to link a breeding programme to previous activities.

We wish to establish an initial low-key breeding effort. This will act as an initial proof of principle and establish the necessary linkages to allow for a long term coordinated effort to develop new bambara groundnut varieties and varietal mixes. These will increase yields needed for commercial farming without compromising the environmental resilience needed by subsistence farmers at the other end of the spectrum.

In the longer term, we see the emphasis moving from coordination by the University of Nottingham towards linked National programmes with UoN, primarily at our Malaysian Campus, providing technical and training support. This will also integrate into the new Crops for the Future (CFF) international centre for underutilised crops and the recently announced Crops for the Future Research Centre (CFF-RC) with a Malaysian government grant of \$38 million to cover buildings, infrastructure and staff for the next seven years. The former has a remit to disseminate best practice and to promote underutilised crops as a joint venture between Bioversity and University Nottingham Malaysia Campus (UNMC), while the latter can provide dedicated facilities and expertise to support research efforts.

Partners and basic roles

The University of Nottingham at the UK and Malaysian campuses will coordinate activities and provide molecular and controlled environment support. UoN will develop initial, speculative, trait-based crosses and also end-user directed crosses for testing under field conditions and will transfer breeding techniques to the in-field Partners during the course of the programme.

The breeders in Indonesia and Ghana will develop material through to varieties in multiple field sites and contrasting environments and also select directly within their own landrace germplasm for multiplication and release of interim varieties. CFF and CFF-RC will work to disseminate and implement results more widely beyond the current programme.

The starting point

Alongside BAMLINK, Nottingham has been creating new crosses between bambara groundnut landraces (African x African, Indonesian x Indonesian and African x Indonesian) to combine a number of complementary traits and to understand the genetic control of those traits.

Traits for improvement:**1. Photoperiod sensitivity for pod-filling (and flowering)**

We are in the process of introgressing the photoperiod insensitive genotypes identified in BAMLINK into a number of African landraces. Photoperiod sensitivity for pod-filling (and to some extent, flowering) has a profound effect on yield in bambara groundnut when grown in countries away from the equator. It has been suggested that a mismatch between the timing of rains and day-length at flowering/podding makes yields unpredictable. One explicit aim of this breeding programme is to introgress and evaluate material with greater photoperiod insensitivity.

2. Drought tolerance

In Africa and semi-arid regions, the greatest advantage of bambara groundnut is drought tolerance. We are currently creating crosses between a range of drought tolerant and less drought tolerant landraces to begin to unravel the mechanisms involved and to provide material for selection with altered drought tolerance for Ghana to test for development of varieties. Ghana has a range of agro-ecological zones which allow this to be done. However, we would also seek partners in arid regions to be involved in evaluation of this material and the International Institute for Tropical Agriculture (IITA) have already expressed a willingness to be involved.

3. Days to maturity

Multiple African accessions have been trialed in an Indonesian environment and a number have been identified with useful traits for that environment. In particular, Indonesian accessions have late maturity (5 months to harvest), meaning that they do not currently fit into the standard Indonesian cropping cycle. African material with shorter *days to maturity* in an Indonesian environment has been identified and crosses made to try to introduce this modification into the Indonesian germplasm.

We have already developed a number of inbred unselected varieties through Single Seed Descent with a number of candidates for testing in Botswana (PhD student who will return to Botswana to take this further) and our Ghanaian partners have identified five landraces with potential for release after further testing. Importantly, our molecular analysis has established that seed derived from a single plant is essentially an unselected genetically uniform variety. On the basis of this, we have been able to modify both our experimental and breeding approaches, to allow us to evaluate pure material and also make multiple crosses between identical genotypes to yield large numbers of offspring for breeding.

We have also gained considerable background genetic information on one quarter of the germplasm accession held by IITA (500 accessions) through DART and SSR analysis and well as an understanding of the physiological basis of a number of important traits.

Outline of an international breeding programme

We wish to use the resources developed within BAMLINK to work with breeding Partners in Africa (Ghana) and Indonesia to develop improved varieties and variety mixes adapted to a range of environmental conditions, represented by the in-field Partners. Selection of the same materials in the three eco-physiological environments available to each Partners will generate highly valuable comparative breeding data and introduce novel useful genes into breeding selection in Ghana (from African and Indonesian material) and into Indonesia (from African material). Once suitable lines have been developed and characterised, varieties will be released through the in-field partners and we will approach global seed actors, such as the charity arm of Pioneer Hi-Bred who have expressed an interest in involvement.

BAMLINX
Part B Section B2

From this initial base, we will approach other institutes and organisations which have expressed interest, including the Kirkhouse Trust who have breeders in India who would be in a position to help and the International Institute of Tropical Agriculture, who have also offered assistance.

To be able to take the final step and fully integrate from basic research through to field, end-users and value added projects, we need a breeding programme to permit bambara groundnut to make real impact and to complete its development as an exemplar, which will help to guide the work of CFF and CFF-RC.

SECTION 3: Links with Global Crops for the Future

The 2nd International Conference on Underutilised Crops.

This 2nd International Symposium on Underutilised Plant Species followed the, very successful, 1st International Symposium "Underutilised Plant Species for Food, Nutrition, Income and Sustainable Development" held in Arusha, Tanzania in 2008. The Symposium was organised under the auspices of the International Society for Horticultural Science (ISHS) with support from the ISHS Working Group on Underutilised Plant Genetic Resources, the ISHS Commission on Plant Genetic Resources and the ISHS Section on Tropical and Sub-Tropical Fruits. The main organiser of the meeting was the University of Nottingham, Malaysia Campus which, together with the Asia Pacific Oceania Office of Bioversity International, hosts the global Crops for the Future Centre in Malaysia.

Crops for the Future – Beyond Food Security, emphasised the potential role of underutilised plant species to contribute to global food security and nutrition, buffering against the consequences of climate change and increasing agricultural biodiversity.

The Symposium was organised around five main areas that, together, identified approaches and methodologies used in research for the development of underutilised plant species viz.:

1. Nutritional, food-processing and end-user values
2. Economic and marketing potential
3. Physiology, agronomy and agroecological potential
4. Biotechnology
5. Strategic approaches for research and development

Participants shared and discussed strategies to maximise knowledge acquisition, minimise duplication of efforts and identify priority areas for further research and development.

Programme: Five main sessions

Nutritional, processing and end-user values

This session discussed the role of underutilised plant species in contributing to global food security and nutrition. In particular, emphasis will be given to indigenous and scientific studies on the nutritional and processing value of species, product development and approaches for developing novel products and uses.

Economic and marketing potential

Underutilised plant species have local or regional importance but generally lack international recognition. Contributions to this session described case studies that have analysed the potential of underutilised plant species and their products for local and international markets.

Physiology, agronomy and agro-ecological potential

To understand the potential of a species in its current niche and beyond, it is crucial to analyse its agronomic potential and to understand the environmental constraints to its wider cultivation, and how these might be overcome. This session explored the role of underutilised plant species as buffers against climate change. What are the crops for the future climates?

Biotechnology

Advances in biological sciences have brought about a number of biotechnological tools that can be useful in research and development of underutilised plant species. These tools offer alternative opportunities to make rapid progress in the improvement of the germplasm. This session discussed practical applications of biotechnology in research and development of underutilised plant species.

Strategic approaches for research and development

Resources available for research are limited and therefore investment of resources on any underutilised plant species calls for a common methodology that can be applied across a range of underutilised plant species. This session examined strategic approaches that can be used in research for development across many underutilised plant species, and where these can also be used to provide a basis for comparison with major crop species.

Links with Global Crops for the Future Research Centre (CFFRC)

Crops for the Future Research Centre (CFFRC) is the first-of-its-kind global centre for research and development of underutilised plants for food and non-food uses. It will operate as the research arm of Crops for the Future (CFF) hosted in Malaysia by ~~Bioversity International~~ ^{Ca.} and University of Nottingham Malaysia Campus (UNMC).

Co-hosted by the Government of Malaysia and UNMC, CFFRC is a unique public/private partnership between a national government and an international research university. CFFRC is incorporated under the Malaysia Companies Act, guaranteed by the Government of Malaysia and the University of Nottingham but is an independent entity with the freedom to drive innovative methods of research within the wider objectives of CFF and its stakeholders. CFFRC will operate through a Board of Directors that includes representatives from its Guarantors, CFF and an Independent Chair. An international Advisory Board will be established to help guide strategic direction and new initiatives.

CFFRC will establish Malaysia as a global hub for research and knowledge transfer on underutilised crops. In addition to new purpose-built laboratory and field research facilities and a botanical garden of alternative crops, CFFRC will have access to facilities and expertise at the University of Nottingham in Malaysia, UK and China, national agencies such as MARADI, Malaysian universities, CFF, Bioversity International and other international and national agencies, especially in Africa and Asia.

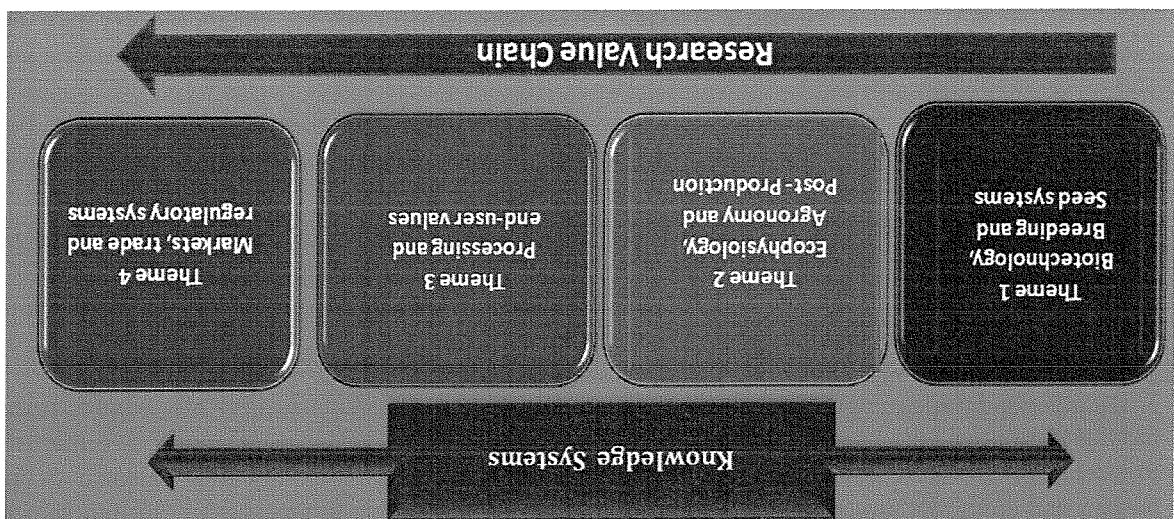
With its partners, CFFRC research will contribute to:

- Improved novel food and non-food crops with nutritional and/or market potential
- A world research centre accessible to local and international researchers and institutions
- Promoting academic and student research programmes in higher learning institutions
- Attracting national and international support for research on potential crops of the future
- Helping make Malaysia a pioneer in research, development and marketing of novel crops.

The CFFRC 'Research Value Chain'

A small number of 'exemplar' underutilised crops will be selected for detailed investigations using a 'Research Value Chain' approach that combines activities across all four research themes and integrated knowledge systems and provides a generic methodology that can be applied to other underutilised crops.

The exemplar species will be identified after an independent review process that links stakeholders that include growers, consumers, researchers and policy makers.



Summary

Throughout the process of negotiations for the establishment of CFFRC and support amounting to \$38 million from the Government of Malaysia, EU funded research on bambara groundnut and the specific example of BAMLINK was used as supporting evidence and justification. The concept of a CFFRC 'Research Value Chain', covering molecular technologies through to end-user benchmarking, is based entirely on the original approach established through BAMLINK and is now set to become the basis for the selection and improvement of exemplar crops through international projects. Further, bambara groundnut is accepted as the role model for these exemplar crops and is likely to become the CFFRC 'standard exemplar crop' against which the development of other underutilised crops will be compared.

As a result of these major international developments, ongoing research on bambara groundnut is envisaged, ideally to be co-ordinated through BAMBREED and links with the FAO AQUACROP suite of models.

The development of AQUACROP, BAMBREED and most importantly CFFRC will provide the maximum possible dissemination of BAMLINK outputs, fulfilling the objectives of all Activity Types in BAMLINK but most significantly alternative mechanisms to achieve the deliverables identified in AT4.

Appendix 1: GRASP

Recent presentation on the Geospatial Resource for Agricultural Species and Pests "GRASP". Once implemented data generated in BAMLINK will be entered under 'genotype' with a geospatial tag, which will allow agroecological matching across countries and regions, along with the longer term implementation of climate change scenarios, to allow candidate material for future breeding to be identified.

GRASP

Geospatial Resource for Agricultural Species Pests

HOME DATABASE DOCS TEAM ABOUT

Geospatial Resource for Agricultural Species and Pests (GRASP) is a seed corn project funded by the Food Security Strategy Group of the University of Nottingham.

The key aim of GRASP project is the development of an open source, open standards based framework for geospatial data capture to exploit genetic trait diversity in animal and crop breeding for improving food security. Cross-disciplinary expertise of the project team is used to develop a framework database which will allow knowledge gathering across all subjects relevant to Food Security.

The database object will be (agricultural species germplasm) genotype(s) with the data ordered by geospatial origin and the higher level descriptor being 'agricultural trait'. The open approach (both in terms of datatype and species) will mean that we develop a tool which is potentially relevant to most research groups in the Food Security area and would hopefully lead to the development of additional tools and analyses by non-UoN partners, as well as breeding and research companies.

A successful implementation of this project would form one of the novel approaches currently being developed by Crops for the Future.

Website design by Matthew Butler, 2011.

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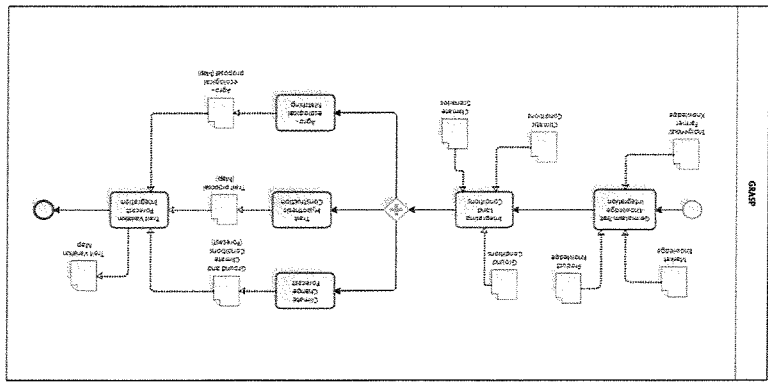
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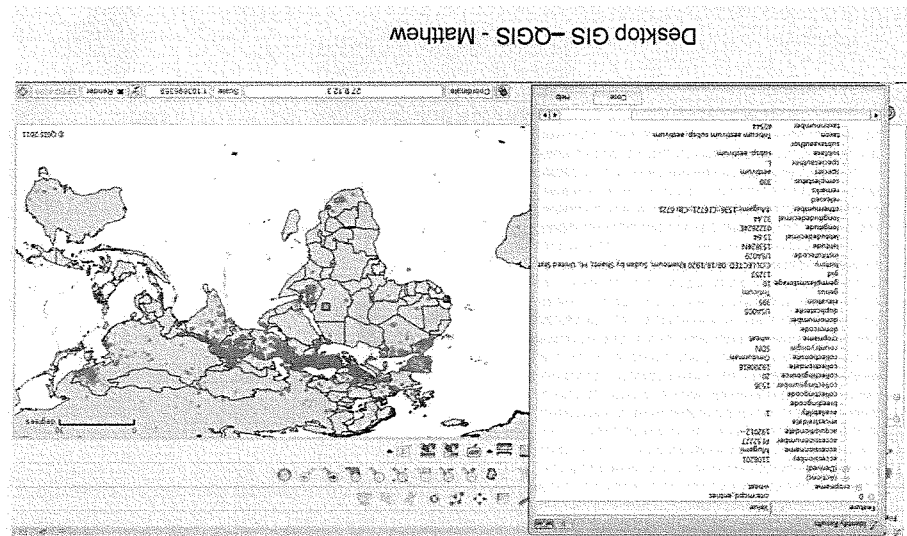
BPMMN - VO




the centre for plant integrative biology




Centre for Geospatial Science



Desktop GIS - QGIS - Matthew



Centre for Geospatial Science



The University of Nottingham

GRASP server

- grasp.nottingham.ac.uk
- O/S: OpenSUSE 11.2
- Memory: 4Gb
- Hard Disk: 100Gb

Java 1.6 (SDK+JRE)

Apache 2

TomCat 7 (disabled)

PostgresPlus 8.4

PostgreSQL 8.4 + PostGIS

R-Patched 1.8

GraspServer80 1.0 (

GEOS 3.3

Proj4 (4.7)


ActivePerl 5.12

GeoTools 2.7.1


LibXML2 (2.7.8)

JDBC 8.4 + 9.0


TCL+TK



Centre for Geospatial Science



The University of Nottingham



Geoserver

GRASP: Species

List of Species known to Geoserver. You can choose the authority, filter based on the name and description, and gather details on each species

Search

name

description

It's a nice ~

This is wheat ~

What?

Results 1 to 2 (out of 2 items)

<< < > >>

GRASP: Order

GRASP: Species

Demos

☐ Layer Preview

Data

☐ About Geoserver

About & Status

username

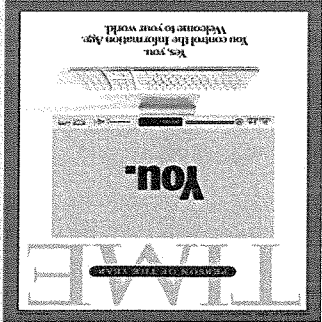
password

☐ Remember me

[illegible]

GRASP - Key Message

Building an open source, open data framework for Crowd Sourcing of Data & Information for agricultural research



Represents the individual content creator on the World Wide Web

- Scalable
- Interoperability
- Low costs
- No proprietary lockin
- Benefits wider community
- In synergy with CFF mission to support, collect, synthesize and promote knowledge on neglected and underutilised species for the benefit of the poor and the environment

APPENDIX 2: Papers presented at the KL conference

BAMLINK

Part B Section B2

-Developing genetic mapping and marker-assisted breeding techniques in Bambara groundnut (*Vigna subterranea* L.) Nariman Ahmad^{1*}, Shrivani Basu¹, Endah Sri Redjeki¹, Erik Murchie¹, Sayed Azam-Ali², Andrzej Kilian³ and Sean Mayes¹

¹Plant and Crop Sciences, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK; ²University of Malaysia Nottingham Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia; ³DART Pty Ltd., 1 Wilf Crane Cr., Yarralumla, ACT 2600, Australia

*Corresponding author: Sbxdna3@nottingham.ac.uk

Keywords: Bambara groundnut, Genetic map, microsatellite, DART, QTL, breeding program

ABSTRACT

Bambara groundnut (*Vigna subterranea* L. (Verdc.)) is an underutilised African legume crop which shows a high degree of drought tolerance. It continues to be an important leguminous crop in tropical Africa and is grown mainly by women who are small-scale farmers in Africa under traditional low input agricultural systems. It is grown mainly for its protein and carbohydrate, as a fixer of agricultural nitrogen and for its high levels of drought tolerance. Genetic studies into this species could provide important data for breeding programmes and to enhance food security in sub-Saharan Africa. The main project work was to construct two genetic linkage maps for bambara groundnut and to carry out a trait QTL analysis. Genomic DNA was extracted from 73 bulked lines (effectively F₃) derived from a cross between the Tiga necara and DipC landraces. A set of 94 pre-tested SSR primers designed from a 454-sequenced microsatellite-enriched library were tested for segregation in this cross. Thirty primers were polymorphic and revealed that the residual heterozygosity in this population was around 25%, consistent with an F₃ population. DART markers for bambara groundnut were previously developed and have also been applied in this study. The results of mapping and QTL analysis will be presented. A second cross between a domesticated landrace (DipC) and a non-domesticated accession (VSSP11) was mapped based on AFLP, DART markers and limited numbers of SSR markers. The second cross allowed an analysis of domestication traits in this species, while the Tiga necara x DipC cross revealed factors of potential importance for breeding programmes. The development of molecular tools potentially allows more rapid breeding progress in this species to be achieved and results so far will be presented.

INTRODUCTION

Bambara groundnut (*V. subterranea* L. Verdc., $2n=2x=22$) is an underutilised African legume crop. It continues to be the third most important minor food legume crop in semi-arid Africa, after groundnut and cowpea. Bambara has undergone a prolonged period of adaptation to drought, pest and disease resistance through low input agriculture (Azam-Ali, 2001). It is a rich source of protein and along with other local sources of protein could help to alleviate nutritional problems in some areas (Massawe, 2005). Despite its importance as a part of the diet of much of sub-Saharan Africa, there are no established varieties of bambara groundnut and the crop is still cultivated from local landraces rather than as varieties. Although, Bambara groundnut is characterised by higher genetic diversity in the wild ancestor, many landraces also consist of multiple genotypes which potentially increases the tolerance of the landrace to the biotic and abiotic stresses. Both wild material and genotypes within landraces could represent an important potential source of beneficial genes for bambara groundnut breeding programs.

Genetic markers work as indicators for the presence of the desirable allele of the target gene, also revealing differences between genotypes. One of the main uses of DNA markers is the construction of linkage maps in crop species to identifying chromosomal regions that contain genes controlling simple traits and quantitative traits using QTL analysis (Mohan *et al.*, 1997). Detecting association

between the phenotype and the genotype of the markers involves dividing the population into different groups depending on a particular marker locus and testing for significant differences between groups with respect to the trait being measured (Tanksey, 1993; Young, 1996). Ideally, all the QTLs selected for MAS should be stable across environments (Hittalmani *et al.*, 2002; Ribaut and Betran, 1999). In addition, the reliability of these markers must be adequately tested for prediction of phenotype before they are used in MAS.

SSRs (simple, tandemly repeated di- to tetra-nucleotide sequence motifs flanked by unique sequences) have been developed in many crop plants (Phillips *et al.*, 2001; Varshney *et al.*, 2004). Furthermore, they are valuable as genetic markers for different applications in plant genetics and breeding, because they are co-dominant, detect high levels of allelic diversity, and are easily and economically analyzed by the polymerase chain reaction (PCR; Susan *et al.*, 1997; Powell *et al.*, 1996). In addition they are effective for investigating phenotypic and genotypic variation within plant species (Gupta and Varshney, 2000).

Diversity Arrays Technology (DAT) is a hybridization microarray platform which is a generic and cost effective genotyping technology. It is a suitable technique for genome-wide discovery and genotyping of genetic variation. DAT allows the simultaneous scoring of thousands of restriction site based polymorphisms between genotypes and does not require DNA sequence information or site-specific oligonucleotides (Alexander *et al.* 2005).

The objectives of the present study were to construct and develop a genetic linkage map of bambara groundnut, combining microsatellite and DAT markers using the segregating population from an intra-specific cross of DIPC and Tiga necara landraces to identify marker-trait linkages, to increase the availability of marker pools available to breeders and to develop this crop through marker-assisted selection, as a part of conventional breeding.

MATERIALS AND METHODS

Mapping population and genomic DNA extraction

The segregating F₃ population seed derived from an intra-specific cross between the Tiga necara (female) and DIPC (male) landraces received from IITA were used as a mapping population. The total genomic DNA was extracted from all 73 lines of segregating population using the Dellaporta protocol (1983) with modification. DNA collected was resuspended in approximately 500 µl of TE and stored at -20°C. DNA integrity and purity were tested using agarose gel electrophoresis with ethidium bromide staining and visualisation under UV light.

The field trial was carried out in Indonesia in 30th May 2010 for all 73 F₃ lines. They were planted in rows distanced 40 cm between them and 40 cm between the plants in the row. The traits were measured according to descriptions in the book 'Bambara groundnut (*Vigna subterranea*)' (IPGRI, 2000). The data was recorded for Descriptors for Bambara groundnut (*Vigna subterranea*) (IPGRI, 2000). The data was recorded for the individual plant at different growth stages and at maturation. Previously recorded data from Swaziland from the F₂ segregating population of the same cross were also used for QTL analysis.

Anderson Darling tests (Stephens, 1974) were used to test the distribution of trait data (Table 1). The inheritance and segregation of contrasting morphological traits were studied in the segregated population of DIPC and Tiga necara landraces. Statistical software MINITAB (Release 16) was used to analyze the variance, constructing residual plots and detecting significant association between the traits ($p < 0.05$). Also phenotypic variation was estimated using regression analysis.

Microsatellite assay

A total of 94 primers derived from a 454-sequenced microsatellite-enriched library were screened for polymorphism and variable loci were amplified from the segregating population. The primers were designed using the Primer3 web program version 0.4.0 (<http://frodo.wi.mit.edu/primer3/>) by Steve Rozen and Helen J. Skaletsky (2000). PCR reactions were carried out in 20 µl reaction volumes under the following conditions: Initial denaturation 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 50-60°C for 1 min (at the optimal annealing temperature derived from a gradient annealing temperature PCR reaction) and 72°C for 2 min, followed by a final elongation step of 72°C for 10 min. The PCR products were run

on 2% agarose and visualized. The CEQ^M 8000 Fragments Analysis Software Version 8 (Beckman Coulter Inc., Fullerton, USA) was used to measure and analyse the fragment sizes of the PCR products.

DART assay

Diversity array technology marker assays were performed by DART Pty. Ltd (Yarralumla, Australia; www.diversityarrays.com) as previously described (Wenzl *et al.* 2004; Akbari *et al.* 2006; Semagn *et al.* 2006).

Construction of linkage map and QTL mapping

JoinMap4 software (Van Ooijen, 2006) was used to construct the linkage map, comprising of both SSR and DART data. Phase determination was carried out by analysing the population initially as a Cross Pollinator (CP) to determine linkage phase for the DART markers, as parental data was not available. Phase in the linkage groups was used to convert DART marker genotypes to different phases in an 'RIL3' population type dataset ({0,0} gave a or c, while {1,1} gave b,d.) RIL loci were grouped according to 'independence LOD', groups were manually selected from LODs 3-5 and Regression mapping carried out. The Linkage map was analysed using the MapQTL version 6 software (Van Ooijen, 2009). Nonparametric mapping of Kruskal-Wallis and IM analysis were both carried out in MapQTL6.

RESULTS AND DISCUSSIONS

SSR markers

A total of 30 SSR primer pairs out of 94 were identified as polymorphic and were mapped. Previously, a low level of genetic polymorphism in the specific gene pool of bambara groundnut had been reported (Basu, 2005). Levels of heterozygosity in the population were found to be approximately 26% using polymorphic SSR markers. This is consistent with this population being an F3.

DART Marker

Of the 7500 fragments detected in DART array, 236 (3.1%) were identified as polymorphic markers in the cross and scored in this population. A total of 266 polymorphic loci were used to assemble the genetic linkage map, including 30 SSR and 236 DART markers. The resultant map consists of 231 linked markers, a total of 498.3 centimorgans with 25 linkage groups of two or more markers.

Segregation distortion

Segregation distortion of the markers was found to be high at 31.6%. SSR markers showed less distortion of only 16.7% in comparison to the DART markers. No information about the original parents of this population and some missing data, especially in DART locus analysis, had an influence segregation distortion. Previous investigations have been reported 40.6% distortion in an F2 intraspecific population of *Medicago torvalia* (Janczewski *et al.*, 1997). One of the highest frequencies of marker distortion (73%) was reported in an interspecific recombinant line population in tomato (Xu *et al.* 1997).

Distribution of markers

The 25 linkage groups spanned 498.3 cM of the bambara groundnut genome (Table 2). The distance between two consecutive markers varied from 0-16 cM, with a mean of 2.71 cM. The longest group in cM was 13 spaced markers, covering a distance of 76.4 cM. The largest number of 40 markers was in linkage group 1, spanning 58 cM, although the clustering of markers on this group perhaps suggests a repeat cluster among the DART markers (Fig. 1).

Quantitative Trait Loci (QTL) mapping

Association of the traits

A Pearson's correlation analysis was conducted to determine the association between the traits of study. In the F3 population seed weight/plant was found to be associated with both pod and internode length, with correlation coefficients of +0.411 ($p = 0.001$) and +0.392 (P -value = 0.003), respectively (Table 3). A highly significant correlation was observed between plant spread and leaflet length ($r = 0.770$; $p = 0.000$). Biomass (dry weight) was correlated positively to both node no./plant and internode length explaining of 37.25% and 13.3% of the trait variation ($p = 0.000$ & 0.003), respectively.

An association was also found between a number of traits in the F2 segregation population. A significant positive correlation was observed between leaf no./plant and plant spread ($r = 0.645$, $p = 0.000$), regression analysis between the two traits explained 40.8% of the variation (Table 4). The regression analysis suggested that 100 seed weight was associated with 20.3% of the variation in the leaf no./plant. Days to emergence was also correlated with 100 seed weight ($r = 0.308$; $p = 0.007$).

Marker and trait associations

Leaflet length (cm)

A QTL for leaflet length was identified by Kruskal-wallis mapping on the LG 1. The most significant association was found at 22.3 cM, between this trait and marker bgPt-602039, scored $K^* = 17.779$ at $P = 0.0001$ (Table 5). This QTL explained 21.9% of the phenotypic variation (Table 5). The two other putative QTLs located on LG 5 and 14 were associated with SSR markers PRIMER95 and mBam3c033 at significant level of $P = 0.05$ scored $K^* = 7.25$ and 8.32, respectively.

Pod length (mm)

A single QTL for pod length was identified by IM analysis located on LG 14 at 10.1 cM, at a LOD score of 3.27 (Table 5). This genomic region explained 21.3 % of the phenotypic variations. The two markers of mBam3c033 and bgPabg-594305 were candidate to flanking this QTL region at 8.95 and 11.81 cM locations, valued 2.93 and 2.98 LOD score, respectively ($K^* = 14.63$ & 12.27 at $P \leq 0.005$). This loci explained 21.3% of the phenotypic variation in pod length in this present study. The locus mBam3c033 was also found to be associated with leaflet length.

Internode length (mm)

A major QTL on LG 3 mapped by IM at 3 cM at a very high LOD score of 7.09 associated with the bgPabg-596988 marker explained 40.9 % of phenotypic variation and also showing a strong association in a nonparametric mapping ($K^* = 20.927$ at $P = 0.0001$) (Table 5)

Node No./stem

A single QTL for node no./stem was identified associated with the marker bgPabg-597746 at 30.2 cM, at 2.78 LOD on the LG 2 ($K^* = 11.86$ at $P = 0.001$) (Table 5). This QTL explained 12.3% of the phenotypic variations for the node no./stem.

Plant Spread (cm)

A putative QTL was identified on LG 1 at 22.3 cM with a LOD score of 2.36. This genomic region explained 15.4% of the phenotypic variation for plant spread in the F3 progenies of this cross. The marker locus bgPt-602039 was found to be associated with this trait ($K^* = 9.347$ at $P = 0.005$).

Seed No./plant

On LG 13 another putative QTL was identified 15.8 cM with interval mapping, at a LOD of 2.48 which is below the significant level of threshold ratio (tested by running 10000 permutation tests). This genomic region explains 17.6% of phenotypic variations in seed no./plant (Table 5).

Date to emergence
The Kruskal-Wallis analysis indicated a significant association between date to emergence and the markers bgPt-595387 and PRIMER16 on the LGs 6 and 19, respectively (Table 5). The co dominant marker SSR loci PRIMER16 was found to be the most significantly linked ($K^* = 10.67$ at $P = 0.005$) loci to the putative QTL for this trait.

Leaf No./plant

The leaf no./plant was found to be affected by the major QTL with LOD score 3.11 for the marker loci bgPt-598428 on the LG 8 ($K^* = 8.123$ at $P = 0.005$). This QTL region explained 17.8 of the phenotypic variations observed in this studied trait.

In order to obtain more reliable QTLs for the agronomical important traits a single QTL-model analysis of Interval Mapping, was used to tag the QTL locus for all the traits except for leaflet length and date to emergence which show non-normal distribution of the data. Although the Kruskal-Wallis test is a primarily detection of Marker-QTL associations (van Ooijen and Mahépadar, 2001), we used a significance threshold at $P < 0.005$ to identify the QTL for traits leaflet length and date to emergence to reveal three QTLs on LGs 1, 6 and 19 at a confidence level of 0.95. However, the population of study here was not big enough to construct a high resolution map for QTL study (Collard *et al.*, 2005) Additional work is required to evaluate the population under different environmental conditions, involving more polymorphic markers and a better understanding of agronomical complex traits to confirm these QTL markers to be used in marker assistant selection of bambara groundnut crop.

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Tables

Table 1. Statistical analysis and the distribution of traits in the two populations of study

F ₃ progenies									
Trait	Min. value	Max. value	Median	Mean value	Variance	Skewness	Kurtosis	Individual No	Distribution
Leaflet length(cm)	2.03	6.05	5.03	4.84	0.70	-1.32	1.84	65	Non-normalized normal
Pod length(mm)	8.00	20.00	13.51	13.61	3.14	0.66	3.75	63	normal at 99%
Node no./stem	4.00	31.00	14.08	14.59	15.37	0.93	4.30	63	normal
Internode length (mm)	2.80	22.40	9.00	10.12	18.31	0.66	0.27	62	normal
Plant spread (cm)	2.60	41.18	27.75	27.06	76.60	-0.68	0.57	65	Normal
Seed no./plant	1.00	20.40	6.83	6.88	17.51	0.72	0.46	59	normal
F ₃ progenies									
Days to emergence	1.456	790.046	0.662	1.456	16.707	0.818776	0.629145	76	Non-normal
Leaf no./plant	1.456	790.046	0.662	1.456	790.0456	0.469311	-0.16038	76	Normal

Table 2. Distribution of the markers, length of linkage groups and marker density in the genetic map constructed with F₃ population of Tiga necara X DipC cross.

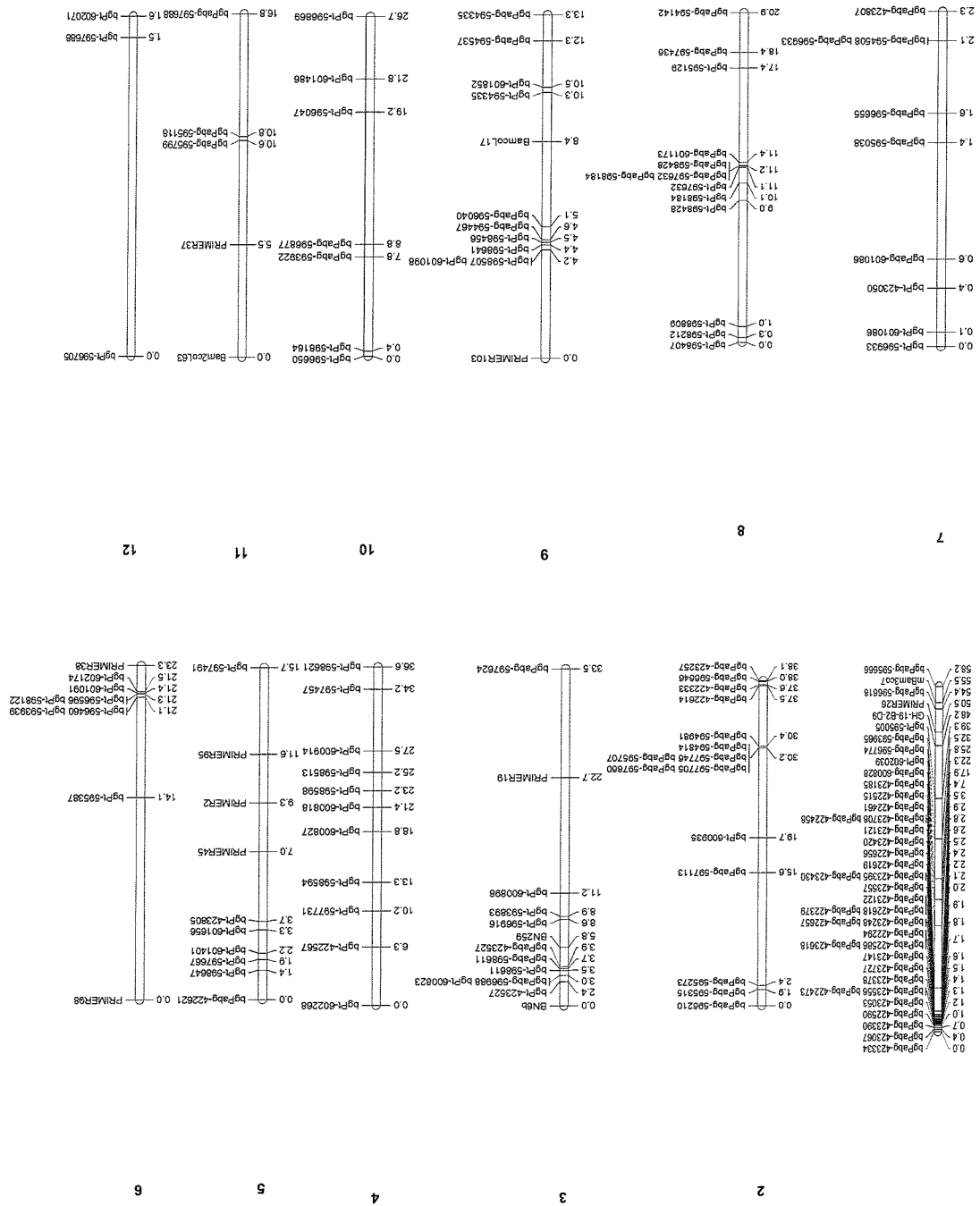
Linkage groups	Length (cM)	No. Of markers mapped in the groups			Mean distances (cM/marker)
1 (Map 2)	58.2	40	3	37	1.46
2	38.1	15	0	15	2.54
3	33.5	13	3	10	2.58
4	36.6	11	0	11	3.33
5 (Map 3)	15.7	10	3	7	1.57
6 (Map 2)	23.3	9	2	7	2.59
7	2.3	9	0	9	0.26
8 (Map 2)	20.9	13	0	13	1.61
9	13.3	12	2	10	1.11
10	26.7	7	0	7	3.81
11	16.8	5	2	3	3.36
12	1.6	3	0	3	0.53
13	76.4	23	4	19	3.32
14 (Map 3)	11.8	15	1	14	0.79
15	3.1	9	1	8	0.34
16	9.4	8	0	8	1.18
17	19.1	6	0	6	3.18
18	8	5	1	4	1.60
19	23.5	4	2	2	5.88
20	18.9	4	1	3	4.73
21	2	2	0	2	1.00
22	5.1	2	1	1	2.55
23	0	2	0	2	0.00
24	32.6	2	0	2	16.30
25	1.4	2	0	2	0.70
Total	498.3	231	26	205	0.26-5.88
Range	0-76.4	2-40	0-4	1-37	0.26-5.88

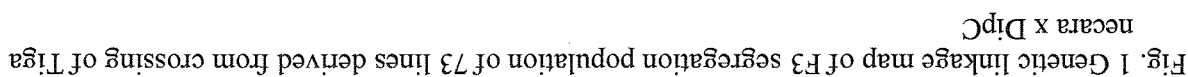
Table 3. Pearson correlation coefficient between the traits in the F₃ population of the cross between Tiga necara X DipC

e locati	group on	(cM)	K*	nec level	mapping (cM)	cc	Explaine d
Leaflet	1	22.31	bgPt-602039	17.79	1	*****	
length	14	8.95	mbam3co33	8.324	2	**	2.4
	5	11.64	PRIMER95	7.251	2	**	
Pod length	14	8.95	mbam3co33	14.629	2	*****	10.08
	14	11.81	bgPabg-594305	12.274	1	*****	3.27
Internode length	3	2.98	bgPabg-596988	20.927	1	*****	0.04
node no./stem	2	30.20	bgPabg-597746	11.862	1	*****	2.93
plant spread	1	22.31	bgPt-602039	9.347	1	*****	0.12
Seed	3	3.48	bgPt-598611	12.487	1	*****	0.13
no./plant	4	36.64	bgPt-598621	7.884	1	*****	0.89
Days to emergence	19	23.47	PRIMER16	10.671	2	*****	2.53
Leaf no./plant	8	8.96	bgPt-598428	8.123	1	*****	0.95
	6	14.06	bgPt-595387	9.951	1	*****	0.80
	8						0.81

Significant level of K* values: **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, *****: 0.0005, *****: 0.0001

Figure 1 Genetic linkage groups identified in the 'narrow' cross.





-Molecular analysis of bambara groundnut, an underutilised African legume crop as part of the BAMLINK project – what lessons can we learn? Mayes, S.^{1,*}, Stadler, F.², Basu, S.¹, Molosiwa, O.¹, Sri Redjeki, E.¹, Ahmad, N.¹, Chai, H.H.¹, Noah, S.¹, Mayes, K.¹, Massawe, F.³, Moller, V.², Roberts, J.¹, Azam-Ali, S.³

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Bambara groundnut (*Vigna subterranea* L. Verde) is an underutilised, drought tolerant legume which has the potential to form an important part of Food Security for the coming decades. The challenges facing farmers to produce enough food for the growing world population—particularly that of climatic instability – are well documented and together represent probably one of the biggest challenges humanity has faced. Our extreme reliance on a limited number of staple (often non-indigenous and sometimes also poorly adapted) crops represents a clear vulnerability. This can be partly reduced by the development of alternative crops. These currently underutilized crops often have beneficial characteristics not found in conventional main crops and if these traits address either biotic or abiotic stresses in a sustainable way, then there is the potential for diversification. There are a number of stumbling blocks to developing such crops, including; poor yields, unadapted crop features, limited processing knowledge, few value-added products, poorly developed transport chains and markets, negative cultural perceptions and little perceived profit margin for commercial breeders. An integrated approach is needed to begin to address these problems. As part of this, we focus on the application of molecular genetics to bambara groundnut and the opportunities to exploit knowledge from other species, new technologies and new approaches to establish a framework for genetic improvement through breeding. We also try to draw out lessons from our work in bambara groundnut which may be relevant in other underutilised species, to try to contribute to the development of a generic approach (and hopefully, a faster and cheaper approach) to tackling these questions in other underutilized species.

-The effect of temperature and drought stress on radiation use efficiency of bambara groundnut (*Vigna subterranea* (L.) Verdc) landraces Ibraheem Al Shareef, Debbie Sparkes, Asha Karunaratne and Sayed Azam-ali

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Summary

Experiments were conducted at the University of Nottingham, Sutton Bonington Campus, UK, to investigate the effect of drought and high temperature stress on the growth and development of bambara groundnut (*Vigna subterranea* (L.) Verdc). In the first glasshouse experiment (2007), two landraces were grown, S19-3 (from hot, dry environment/ Namibia) and Uniswa Red (from cool, wet environment/ Swaziland) under two different temperatures, $33 \pm 5^\circ\text{C}$ and $23 \pm 5^\circ\text{C}$. Drought was imposed at pod filling stage (77 DAS). In the second experiment (2008), the same two landraces were grown under the same temperatures, but the drought was imposed at flowering (30 DAS). The landraces under both temperature regimes intercepted less radiation in 2008, and Uniswa Red at 33°C had the highest intercepted radiation in both years. The light extinction coefficient was not affected either by temperature or drought; 0.59 in 2007 and 0.57 in 2008. In both growing seasons, fractional intercepted radiation was higher at 33°C . The conversion coefficient for intercepted radiation (ϵ_s , g MJ^{-1}) of S19-3 was significantly higher at 33°C in both growing seasons. The highest value (1.31) was obtained from S19-3 at 33°C in 2007, and the lowest (0.24) was obtained from S19-3 at 23°C in 2008.

Introduction:

Bambara groundnut is a short day crop which grows at elevations up to 1600 m. The optimum temperature for bambara groundnut is 28°C (Linnemann and Azam-Ali, 1993). The landraces S19-3, which originated from a hot and dry environment (Namibia) and Uniswa Red, which originated from wet and cool environment (Swaziland) have been extensively used for several studies at The Tropical Crops Research Unit (TCRU), Sutton Bonington, University of Nottingham. The landraces were chosen as representative landraces of two contrasting environments. A considerable number of studies have been carried out on bambara groundnut to evaluate the effect of drought stress on the fractional intercepted radiation (f) and radiation use efficiency (the ratio of dry matter produced to solar radiation intercepted (ϵ_s). Collinson *et al.* (1999) reported that seasonal f ranged from 0.2-0.37 for droughted bambara groundnut and 0.62-0.74 for irrigated bambara groundnut. The same authors reported ϵ_s 1.00 g MJ^{-1} under non-limiting soil moisture and 0.51 g MJ^{-1} under drought. In a similar study, Mwale *et al.* (2007) reported values of 1.51 g MJ^{-1} and 1.02 g MJ^{-1} under irrigated and drought conditions, respectively.

In a study on the effect of temperature on f and ϵ_s in bambara groundnut, Chickweye (2006) found that landraces at 33°C intercepted more radiation than stands at 23°C . The study reported ϵ_s values of 1.07 g MJ^{-1} and 1.02 g MJ^{-1} for S19-3 and Uniswa Red grown at 33°C , respectively, and 1.01 and 0.74 g MJ^{-1} for Uniswa Red and S19-3 at 23°C . Collinson *et al.* (1999) reported a mean k value of 0.62 for bambara groundnut grown at mean air temperature of 27°C at TCRU. While previous studies have investigated the effect of temperature or drought in isolation, the current study considers the combined effect of heat and drought stress on the radiation use efficiency of bambara groundnut.

Materials and methods

The study was conducted in the Tropical Crops Research Unit (TCRU) glasshouses at the University of Nottingham, UK, during the summers of 2007 and 2008. The TCRU glasshouses have the facilities to control temperature, CO_2 concentration and atmospheric humidity. Full description of the glasshouses is found in Clifford *et al.* (1993). The study involved two landraces, S19-3 from Namibia and Uniswa Red from Swaziland.

Experimental layout and crop management

The experimental design was a split plot with temperature on the main plot (high temperature $33\pm5^{\circ}\text{C}$, low temperature $23\pm5^{\circ}\text{C}$) and landrace (S19-3 and Uniswa-Red) on the sub-plots. In 2007, drought was imposed at podding, 77 days after sowing (DAS) and at flowering (30 DAS) in 2008. All glasshouses received natural daylight, and because bambara groundnut is a short day plant for pod filling, the day length was controlled by covering the crop stands in each plot with a black polythene screen at 00 and uncovering at 00 to maintain 12h photoperiod from 21 DAS until 113 DAS. Atmospheric saturation deficit (SD) was set not to exceed 4 kPa at 33°C and not to exceed 2 kPa at 23°C . The crops were sown at 5cm depth (one seed for each hole) at a spacing of 10 cm between each hole and 35cm between rows. There were 12 rows in each plot and 36 holes, effectively 30 seeds m^{-2} . At 26 DAS the crops were thinned to 18 plants per row (approximately 20cm between plants) which gave an established population of 15 plants m^{-2} .

Radiation measurements

In each plot, incident solar radiation (S_i) was measured using two tube solarimeters located 2.3m above ground level, and radiation transmitted through the canopy (S_t) was measured using two solarimeters at ground level. The incident and transmitted radiation were measured every hour and the intercepted radiation for each day was computed as the sum of the hourly differences between S_i and S_t . The sum of the daily values of the intercepted radiation represented the cumulative intercepted radiation (S_{ci}). Fractional intercepted radiation (f) was calculated as $f = (S_i - S_t) / S_i$. K was calculated from the regression of $\ln(1-f)$ against leaf area index.

Radiation use efficiency (RUE; g MJ^{-1}) was determined from the regression between the accumulated above ground dry matter (g m^{-2}) and the total cumulative intercepted radiation (MJ m^{-2}) estimated from the above and below canopy solarimeters. Mean day light SD values were used as normalising factor in calculating radiation equivalent for the two landraces (Azam-Ali *et al.*, 1994)

Growth analysis

Sequential growth analysis, at two to three weeks intervals, was carried out on 10 plants per plot on nine occasions through the season. Plants to be harvested were pre-determined to avoid selecting plants adjacent to previous harvesting locations. No plants were taken from the two edge rows in each plot to avoid edge effects, nor the central areas where light interception measurements and final harvest were taking place. Leaves and pods were counted at growth analysis. Leaf, stem, and pod dry weights were obtained after oven-drying at 80°C for 48h. The mean of 10 plants for each variable was taken as a representative value for each replicate.

Results

In 2007, Uniswa Red at 33°C intercepted more radiation than at 23°C and more than S19-3 at both temperatures, but in 2008, Uniswa Red at 33°C had the lowest intercepted radiation (Figure 1). The statistical analysis of the total intercepted radiation showed no significant differences in either growing season ($P < 0.05$). The pattern of fractional interception of radiation (f) was similar for the two landraces at 33°C in both seasons, while at 23°C they were different (Figure 2). For Uniswa Red, f at 23°C was lower than for S19-3, but it was the opposite in 2008. In both growing seasons, f was higher at 23°C than at 23°C . The maximum f values for the landraces at 33°C were close to 1, while at 23°C it was less than 0.8. However, there were no significant differences in either growing season. The light extinction coefficient (k) was 0.57 in 2007 and 0.59 in 2008 with no significant differences between treatments in either year.

The amount of total dry matter (TDM) accumulated at 23°C was always lower than at 33°C (Figure 3). Statistical analysis showed no significant difference between the two growing seasons although maximum TDM was 588 g m^{-2} in 2007 and 271 g m^{-2} in 2008.

Radiation use efficiency of the two landraces varied in the respect to temperature in 2007 ($P = 0.001$) and in 2008 ($P = 0.047$) (Figure). In both years, S19-3 at 33°C had the highest RUE and S19-3 at 23°C the lowest. In 2007, RUE of Uniswa Red was similar at both temperatures, while in 2008 RUE was higher at 33°C . Radiation use efficiency (RUE) of bambara groundnut was affected by drought in both temperatures and landraces. There were reductions in the radiation conversion coefficient (e_s) from 2007 to 2008 in all treatment combinations. Uniswa Red had the same radiation conversion coefficient (e_s) at both temperatures in 2007, but for S19-3, e_s at 23°C (0.66 g MJ^{-1}) was

half of the value obtained at 33°C (1.31 g MJ⁻¹). In 2008, the effect of temperature on Uniswa Red was clearer when there was a reduction of 23 % in e_s (0.4 g MJ⁻¹ and 0.31 g MJ⁻¹ at 33°C and 23°C, respectively). When SD values were used as normalising factor in calculating radiation equivalent, differences between treatments became greater, but the ranking remains consistent (Figure 4).

Discussion

In the present study, capture of radiation depends on soil moisture, temperature and atmospheric saturation deficit. As drought was imposed earlier in 2008, but all other treatments were the same, there will be a comparison between 2007 and 2008 to represent well-watered and droughted crops and therefore the interaction between water and temperature.

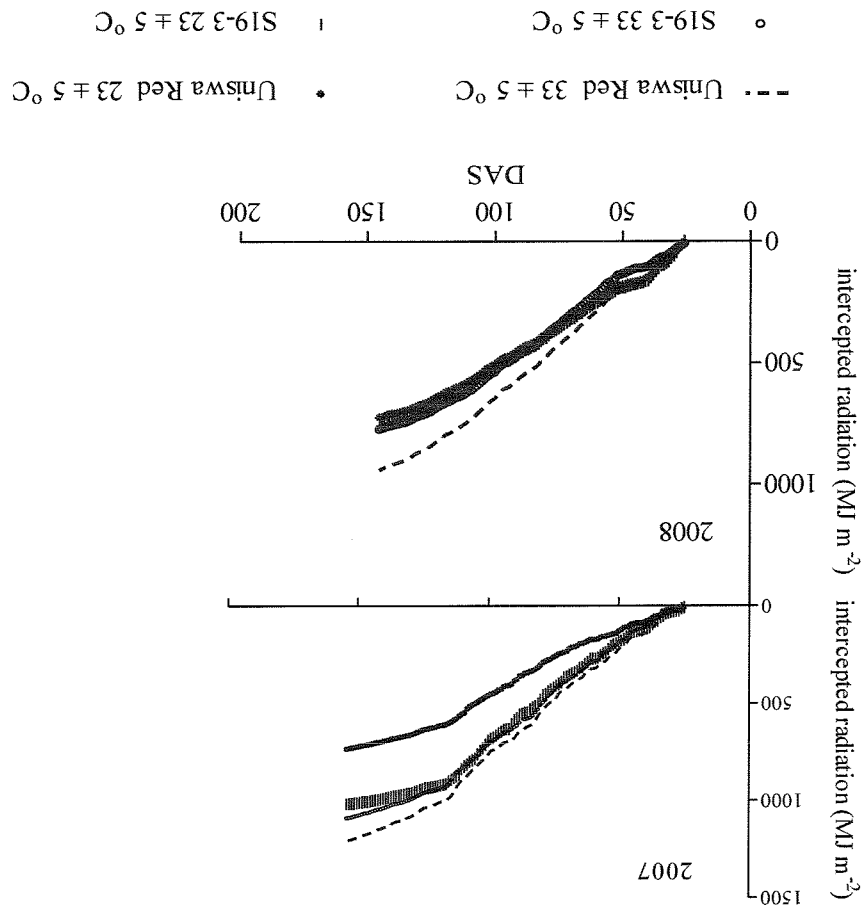
Drought had an effect on the total intercepted radiation (S_{ci}) in the four treatments in both years. The crops intercepted more radiation in 2007 than in 2008 as the drought was more severe in 2008. These results are in agreement with those reported before in bambara groundnut (Mwale *et al.*, 2007) and cowpea (Craufurd and Wheeler, 1999). The fractional interception (f) was similar in 2007 and 2008 which contrasts with Mwale *et al.* (2007) who found that drought reduced f in bambara groundnut. This disagreement could be due to the different temperature and soil water conditions in the present study and Mwale *et al.* (2007), where the crops were grown at 28°C and drought was imposed at 42 DAS.

The intercepted radiation and f were higher at 33°C in both years but the differences between treatments were not significant. f in both years and both temperatures started to decline when the soil moisture decreased by 40 % (data not shown). Mwale *et al.* (2007) reported 30% of soil moisture deficit as a critical value beyond which f was reduced in bambara groundnut, but the comparison between the two studies is not as simple as that; the critical value reported by Mwale *et al.* (2007) was for bambara groundnut grown under optimum temperature (28 °C) and different atmospheric saturation deficit (1-1.6 kPa), and the drought conditions were different in terms of the time that drought was imposed and the amount of water applied to the crops before starting the drought.

For Uniswa Red at 33°C, S_{ci} was reduced from 1200 MJ m⁻² in 2007, when the crop was well-watered to 942 MJ m⁻² in 2008 when drought was imposed early in the life cycle (22% reduction). For S19-3 the reduction was 29%. These values are similar to the differences between an irrigated and droughted crop of bambara (30%) as reported by Mwale *et al.* (2007). The amount of solar radiation intercepted depends not only on the size of the canopy but also on the duration of the foliage. Because of that Uniswa Red, which had longer life cycle than S19-3, intercepted more radiation than S19-3 in both years.

Figure 1 Cumulative intercepted radiation (MJ m^{-2}) of two bambara groundnut landraces (Uniswa Red and S19-3) grown at low temperature ($23 \pm 5^\circ\text{C}$) and high temperature ($33 \pm 5^\circ\text{C}$) in the TCRU glasshouses during the experiments of 2007 and 2008.

Increased temperature increases the rate of canopy expansion in indeterminate crops (e.g. groundnut) but has no direct effect on the duration of canopy expansion or canopy longevity, at least within the limits of the growing season, which are usually determined by factors such as supply of water. Accordingly, the size of the canopy, the radiation interception and the dry matter, at any time after emergence, all increase with rise in temperature between base and optimum temperature (Marshall *et al.*, 1992). In a study on groundnut, Marshall *et al.* (1992) found that f increased with rise in mean temperature from 19°C to 28°C , but when they grew the crop under mean of 31°C f did not increase further. In the present study, maximum f increased with rise in mean temperature from 23°C to 33°C , from less than 0.8 to 0.94. According to Mwale *et al.* (2007), bambara groundnut reached maximum f of 0.8 at 28°C under full irrigation. This might be an indicator that bambara groundnut is different to groundnut, where f can increase further with rise in temperature above the optimum level. This shows bambara groundnut as a crop which has a wide range of ability to grow and develop under conditions of stress.



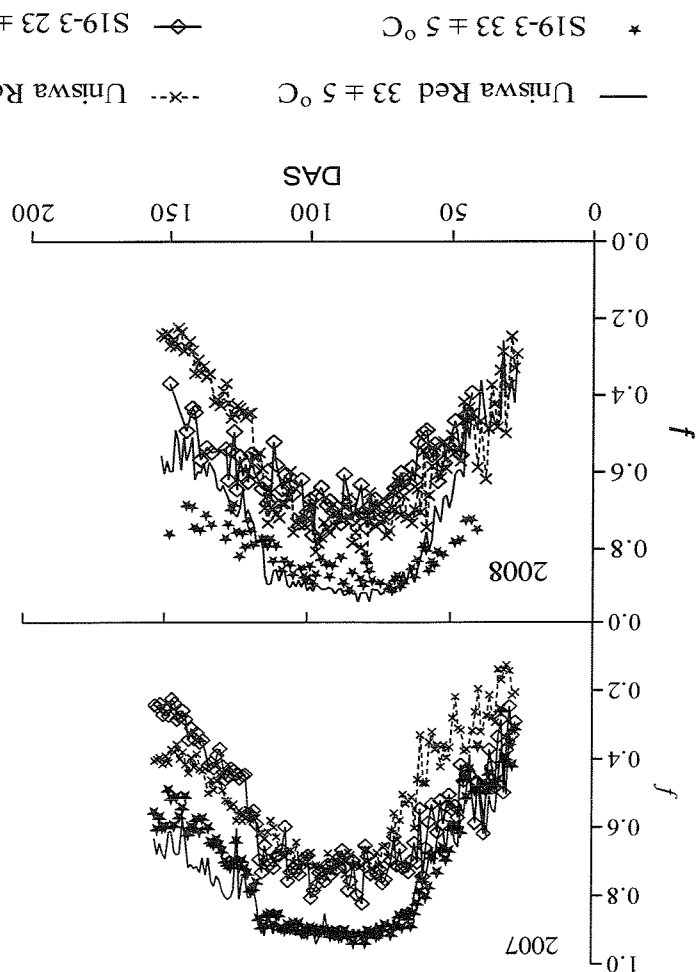


Figure 2 Fractional intercepted radiation of two bambara groundnut landraces (Uniswa Red and S19-3) grown at low temperature ($23 \pm 5^\circ\text{C}$) and high temperature ($33 \pm 5^\circ\text{C}$) in the TCRU glasshouses during the experiments of 2007 and 2008.

In a study on chickpea, bean and cowpea, grown under different soil water regimes, chickpea had the lowest k in all the soil water regimes (Tefaye *et al.*, 2006). The author attributed that to the more horizontal leaves that bean and cowpea had than chickpea. No measurements of heliotropism were carried out in the present study, but it has been noticed that the crops change the leaf angle at the midday, moreover, heliotropism in bambara groundnut in response to drought was reported by Collinson *et al.* (1999). The results of RUE in this study are in agreement with a study by Marshall *et al.* (1992) on groundnut in the TCRU under different means of temperature, reduction in ϵ_s of 30% was found between a stand grown at 31°C (2.13 g MJ^{-1}) and a stand grown at 22°C (1.5 g MJ^{-1}). Throughout the life of the canopy, ϵ_s might be decreased because of the increase in SD. It has been demonstrated that ϵ_s of sorghum and maize is sensitive to SD even under non-stressed conditions (Squire, 1990). In the present study, when SD was used to normalize the amount of water transpired, the difference between the ϵ_s values at high and low temperature increased.

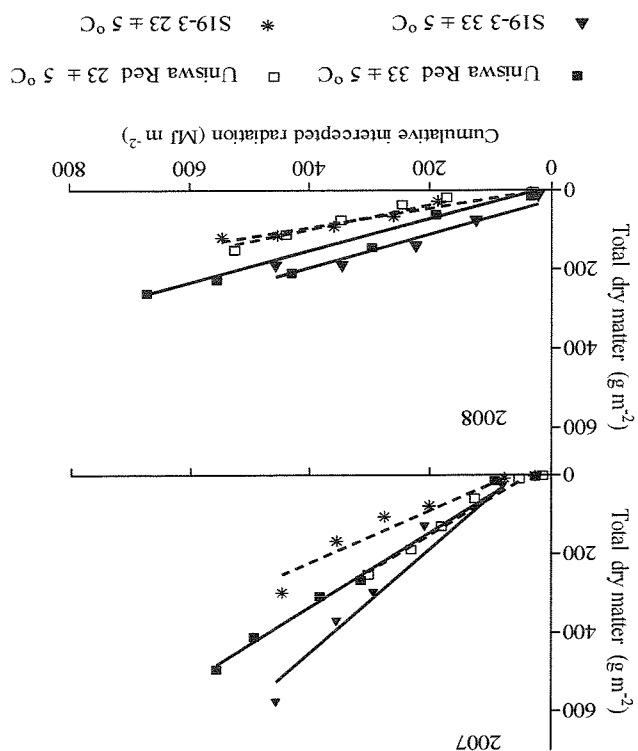


Figure 3 Regression of total dry matter (g m⁻²) cumulative intercepted radiation for two bambara groundnut landraces grown at low temperature (23 ± 5°C) and high temperature (33 ± 5°C) in the Tropical Crops Research Unit (TCRU) during the experiments in 2007 and 2008. For 2007, $r^2=96.1$. For 2008, $r^2=93.9$ Slopes and constants are presented in Table

Table Slopes and constants obtained from the regression of total dry matter (g m⁻²) against cumulative intercepted radiation for two bambara groundnut landraces grown at low temperature (23 ± 5°C) and high temperature (33 ± 5°C) in the Tropical Crops Research Unit (TCRU) during the experiments in 2007 and 2008

	2007		2008		Treatment	
	Slope	Se	Slope	Se	33±5°C	23±5°C
UNI	0.94	0.07	-43.6	25.9	UNI	0.92
UNI	0.13	0.09	-31.3	25	UNI	0.09
UNI	-41.8	25.5	0.42	0.04	UNI	27.43
UNI	0.24	0.04	0.03	0.04	UNI	15.3
UNI	-2.9	16	7.9	15.1	UNI	15.7
UNI	0.66	0.09	0.07	0.13	UNI	1.31
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UNI	0.24	0.04	0.03	0.04	UNI	15.3
UNI	-2.9	16	7.9	15.1	UNI	15.7
UNI	0.66	0.09	0.07	0.13	UNI	1.31
UNI	0.09	0.09	-74.2	25.5	UNI	0.09
UNI	-41.8	25.5	0.42	0.04	UNI	27.43
UNI	0.24	0.04	0.03	0.04	UNI	15.3
UNI	-2.9	16	7.9	15.1	UNI	15.7
UNI	0.66	0.09	0.07	0.13	UNI	1.31
UNI	0.09	0.09	-74.2	25.5	UNI	0.09
UNI	-41.8	25.5	0.42	0.04	UNI	27.43
UNI	0.24	0.04	0.03	0.04	UNI	15.3
UNI	-2.9	16	7.9	15.1	UNI	15.7
UNI	0.66	0.09	0.07	0.13	UNI	1.31
UNI	0.09	0.09	-74.2	25.5	UNI	0.09
UNI	-41.8	25.5	0.42	0.04	UNI	27.43
UNI	0.24	0.04				

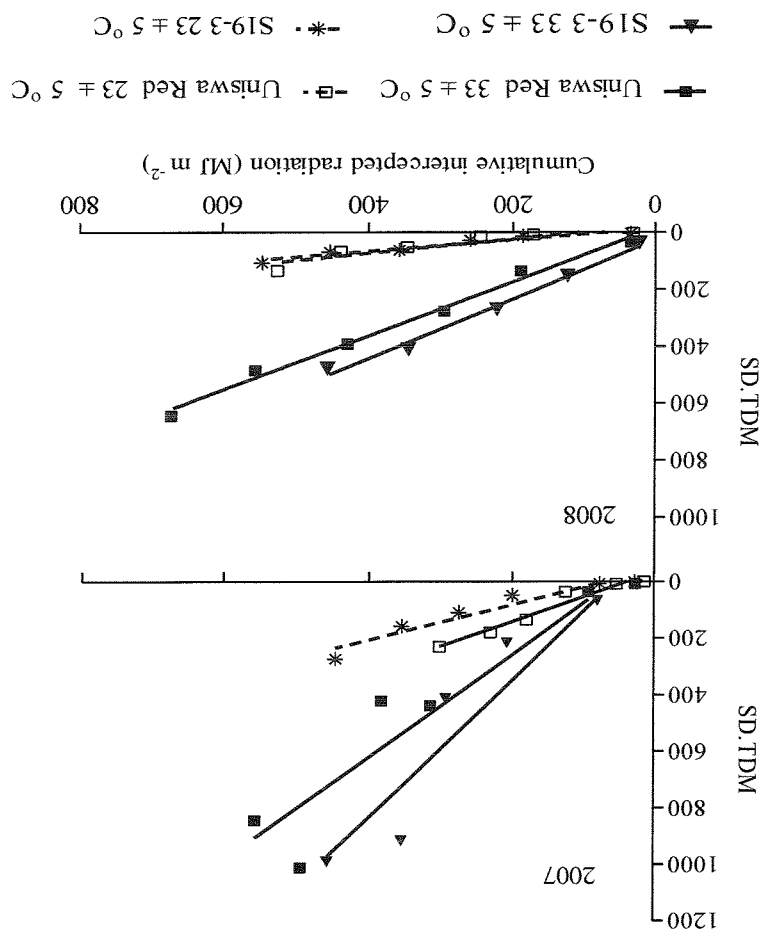


Figure 4 Regression of total dry matter (g m^{-2}) corrected for saturation deficit (SD) against cumulative intercepted radiation for two bambara groundnut landraces grown at low temperature ($23 \pm 5^\circ\text{C}$) and high temperature ($33 \pm 5^\circ\text{C}$) in the Tropical Crops Research Unit (TCRU) during the experiments in 2007 and 2008. For 2007, $r^2=89.5$. For 2008, $r^2=97.7$. Slopes and constants are presented in table 2.

Conclusion

A significant effect of drought on radiation conversion and capture in bambara groundnut was found in this study. S19-3 and Uniswa Red intercepted more radiation and had higher f at the high temperature due to the higher vegetative growth, while temperature did not affect k . High temperature reduced ϵ_s in both landraces.

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Table 2 Slopes and constants obtained from the regression of total dry matter (g m^{-2}) corrected for saturation deficit against cumulative intercepted radiation for two bambara groundnut landraces grown at low temperature ($23 \pm 5^\circ\text{C}$) and high temperature ($33 \pm 5^\circ\text{C}$) in the Tropical Crops Research Unit (TCRU) during the experiments in 2007 and 2008.

	UNI	UNI	UNI	Treatment
	S19-3	S19-3	S19-3	33 \pm 5 $^\circ$ C 23 \pm 5 $^\circ$ C
2007				
Slope	1.81	0.86	2.42	0.62
Se	0.22	0.44	0.29	0.30
Constant	-103.4	-31.3	-136.2	-42.4
Se	83.3	80.5	82	82.1
Slope	0.94	0.25	1.04	0.2
Se	0.03	0.05	0.06	0.05
Constant	-14.5	-26.2	28.1	-13.3
Se	16.7	17.4	17.0	17.6
2008				
Slope	0.94	0.25	1.04	0.2
Se	83.3	80.5	82	82.1
Constant	-103.4	-31.3	-136.2	-42.4
Se	0.22	0.44	0.29	0.30
Slope	1.81	0.86	2.42	0.62

-Effect of Sowing Date on the Performance of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Landraces in the Transition and Forest Agro-ecologies of Ghana. J. N. Berchie¹, H.A. Dapaah¹, A. Agyemang¹, E. Asare², J. Sarkodie Addo², S. Addy¹, J. Addo¹ and E. Blankson¹

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Abstract

Bambara groundnut is an underutilized and until lately, under researched crop. Its ability to produce some yield where other crops such as groundnut fail has been established. The balanced nutritional quality of the crop coupled with its resistance to drought gives it a crop of choice to achieve food security especially in the dry areas of Africa. Experiments were conducted in 2008 in two agro-ecological zones of Ghana namely, Wenchi (Transition) and Fumesua-Kumasi (Forest) agro-ecologies to determine the effect of sowing dates on the yield of bambara groundnut landraces namely; Burkina, NAV 4, NAV Red, Black eye, Tom, Mottled Red and Ada. Sowings were done in a factorial arrangement in a randomized complete block design with three replications. Pod yields ranged between 3.0 t/ha to 4.4 t/ha and 280 kg/ha to 2.3 t/ha for the various sowing dates in the Transition and Forest agro-ecologies respectively. Harvest indices ranged from 0.06 - 0.56. Minor rainy season sowing of bambara groundnut produced more pod yield than major rainy season sowing. Tom was a highly vegetative landrace. Pod yield was highest in the Transition than the Forest agro-ecology. Where irrigation is available, sowing bambara groundnut just before the rains in February and March in the Transition and Forest agro-ecologies of Ghana produce high pod yields.

Key words: Bambara groundnut, Transition agro-ecology, landraces, sowing date

INTRODUCTION

The Transition agro-ecology of Ghana is one of the major bambara groundnut cultivating zones in the country. Not much of the crop is however, cultivated in the forest agro-ecology of the country even though there lies the potential of obtaining some yield when the crop is grown at the appropriate time. Unfortunately however, not much effort has gone into evaluating the performance of bambara groundnut landraces to sowing dates in Ghana. Working on the effect of planting time on growth, flowering and seed yield of nine bambara groundnut landraces, Kumaga *et al.* (2002) observed that moderate rainfall, coupled with relatively high temperatures over the entire growing period, may be required for high pod and seed yield. Sesay *et al.* (2008) observed that the variation in bambara groundnut pod yield across sowing dates was closely associated with variation in pod number per plant, seed size, harvest index and dry matter production. Shegro *et al.* (2010) observed that delayed planting of soybean in the Metekel Zone, North Western Ethiopia resulted in short plants, reduced leaf area and leaf area index. They observed that May and June plantings produced significantly greater grain yields compared to July sowing. Harris and Azam-Ali (1993) attributed the variation in yields of bambara groundnut to the year to year variations in planting dates. Changes in the environmental

factors such as temperature, rainfall, daylength, may greatly affect the growth, reproductive development and the eventual pod yield of the crop.

Sesay *et al.* (2004) noted that the time of planting of bambara groundnut in Swaziland varies from October to January. Within this period however, they observed that changes in the environmental conditions notably daylength and temperature may affect the yield of the crop. Johnson (1968) observed that the timing of planting of bambara groundnut is critical to the yield of the crop. Yields of bambara groundnut in Zimbabwe can reduce from 2530 kg ha⁻¹ to 840 kg ha⁻¹ in a clay soil and 1420 kg ha⁻¹ to 200 kg ha⁻¹ in a sandy soil when sowing is delayed only three weeks (Johnson, 1968). Two studies were therefore undertaken in 2008 to determine whether differences in sowing dates could affect the performance of selected bambara groundnut landraces in the Transition and Forest agro-ecologies of Ghana.

MATERIALS AND METHODS

Seven bambara groundnut landraces; Black eye, Burkina, Tom, NAV 4, NAV Red, Mottled Red and Ada were used for the study. The landraces were sown over six sowing dates; 28/02/08, 13/03/08, 20/03/08, 13/04/08, 17/06/08 and 23/06/08 in Wench. The sowing dates were chosen to simulate changes in duration of day length if any, and environmental changes like rainfall, temperature among others over the growing periods. In Kumasi in the forest agro-ecology, six bambara groundnut landraces were sown on six sowing dates. The landraces were; Black eye, Burkina, Mottled Red, NAV 4, NAV Red and Tom and the sowing dates; 12/03/08, 19/03/08, 14/04/08, 28/04/08, 10/06/08 and 16/06/08. Soil nutrient levels were brought to 50 kg ha⁻¹ N, 25 kg ha⁻¹ P and 25 kg ha⁻¹ K. Sowings were done in a factorial arrangement in a Randomized Complete Block Design (RCBD) with three replications. Seeds were sown at inter-row spacing of 50 cm and intra-row spacing of 20 cm at two seeds per hill and thinned to one plant/hill at 20 days after sowing (DAS) giving a plant population of 10 plants/m². Plot size was 6 m x 6 m (13 rows by 31 hills). Weed control was done by hand-hoeing when weedy. No spraying was done against pests and diseases because pests and diseases have not yet been identified as major problems on bambara groundnut in Ghana. Ten plants were randomly removed at each sampling date at 20 DAS, 45 DAS, 60 DAS, 105 DAS and 120 DAS. These dates fell into the day of thinning, flowering, pod initiation, physiological maturity of some pods and harvesting of pods respectively. Plant dry weights were obtained by placing samples in ovens at 80 °C for 48 hours. Data taken included days to 50% emergence, days to 50% flowering, days to podding, days to maturity, shoot dry weight, immature pod dry weight, mature pod dry weight and total pod yield. Pod harvest index was determined as;

Total pod yield/Total pod + haulm yield. Seed

harvest index (SHI) was calculated as the Total seed yield/Total pod + haulm yield. Data was analysed using a Genstat statistical package. Mean separations were done using the least significance difference

(LSD) at 5% probability level.

RESULTS

Wenchí 2008 Sowing

Days to 50% emergence

Significant differences were observed in days to 50% emergence for the various planting dates and landraces ($p < 0.001$) and the interaction ($p < 0.005$). Ada and Burkina had the earliest number of days to 50% emergence (10.8 and 10.9 days) respectively with Black eye, Tom and NAV Red a little over 12 days (Table 1). The 13/03/08 sowing emerged earliest among the various dates (10.8 days) with the 23/06/08 sowing registering the longest number of days to 50% emergence (12.8 days) (Table 1).

Days to 50% flowering

Significant differences were observed with respect to the planting dates and landraces ($p < 0.001$) and planting dates by landrace interaction ($p = 0.05$) on days to 50% flowering. Ada and Burkina had the least number of days to flowering (37.4 and 37.3 days) respectively and Tom registered the highest number of days to flowering (47.9 days) (Table 1). The 13/03/08 sowing flowered earlier (38.9 days) whereas the 23/06/08 sowings took 42 days to produce 50% flowering (Table 1).

Days to podding

Significant differences were observed in days to podding for the various landraces ($p < 0.001$) and planting dates ($p < 0.001$). There was however, no significant difference with respect to the interaction. Ada and Burkina had the least number of days to podding 66.1 days and Tom the longest number of days to podding 77.2 days (Table 1). The 28/02/08 registered the earliest number of days to podding (67.1) whereas the June (17/06/08 and 23/06/08) sowings gave the longest number of days to podding (69 and 70 days, respectively) (Table 1).

Days to maturity

Significant differences were observed with respect to days to maturity for the planting dates and landraces and the interaction ($p < 0.001$). Ada and Burkina recorded the least number of days to maturity 97 and 96 days respectively. Tom had the longest number of days to maturity (120 days) (Table 1). The 13/03/08 sowing significantly registered the least mean number of days to maturity for all the landraces (97.14) ($p < 0.001$) with the 23/06/08 sowing registering the longest mean number of days to maturity for all the landraces (105 days) (Table 1).

100 pod dry weight

Significant differences were observed on landraces with respect to 100 pod dry weight ($p < 0.001$). No significant difference was observed with respect to planting dates and the interaction with respect to 100 pod dry weight. Tom had the highest 100 pod dry weight (113 g) and Ada and Burkina the least 80.0 g and 79.4 g respectively (Table 2).

Seed dry weight of 100 pods

Significant differences were observed for the planting dates and landrace ($p < 0.001$) and of 100 pods is not equivalent to the 100 seed dry weight since some pods contained more than one seed. Tom obtained the highest seed dry weight of 100 pods (82.8 g) and Ada and Burkina had 53.3 g and 52.2 g respectively (Table 2). Seed dry weight for 100 pods was significantly highest for the 28/02/08 sowing (70.4 g) and least for the 20/03/08 sowing (61.4 g).

Shelling percentage.

Table 3 gives the shelling percentage based on the 100 pod seed dry weights. Shelling percentage was greatest with Mottled Red and NAV 4 (74.1 % and 74.2 %) respectively. Shelling percentage was greatest for the 13/03/08 sowing (79.5 %) (Table 2).

Total dry weight and pod dry weight

Significant differences were observed for planting dates, landraces and the interaction with respect to total pod yield ($p < 0.001$). Black eye registered the greatest total pod yield (4631 kg ha⁻¹) with Ada and Burkina registering 4436 kg ha⁻¹ and 4511 kg ha⁻¹ respectively. Tom registered the least pod yield of 378 kg ha⁻¹ (Table 3).

Table 2 gives the total seed yield based on total pod yield and the shelling percentage. The shelling percentage was rounded up to the nearest whole number. Total seed yield was greatest in Black eye (3381 kg/ha) even though this was not significantly different from the other landraces except Tom which produced the least seed yield (276 kg/ha). Total seed yield was highest for the 13/03/08 (3341.3 kg ha⁻¹) sowing and least for the 23/06/08 (2139.2 kg ha⁻¹) sowing ($p < 0.05$).

Pod and seed harvest indices

Pod harvest index was significantly greatest in Ada and Burkina (0.56) and least in Tom (0.06) ($P < 0.05$). Seed harvest index however, was significantly highest in Mottled Red and NAV 4 (0.41) and least in Tom (0.05) (Table 4). Pod harvest index was significantly highest for the March

2008 Sowing, Kuumasi-Chana (Forest agro-ecology)
Days to 50% emergence

There was no significant difference with respect to landraces and interaction to days of emergence. Significant differences were however, observed with respect to planting dates ($P < 0.001$). The 19th March, 28th April and 1st June sowings emerged earliest (8.2 days) relative to the 13th March sowing (9.4 days) (Table 5)

Days to 50% flowering

Significant differences were observed for planting dates ($P < 0.001$) and landraces ($P < 0.001$) with respect to days to 50% flowering (DFF). NAV Red recorded the least number of days to 50% flowering (42.8 days) and Tom the longest number of days (48.7 days) (Table 5). The 19th March and 14th April sowings took the least number of days to 50% flowering (Table 5)

Days to maturity

There was a significant difference in the number of days to pod maturity for the landraces ($P < 0.001$), planting dates ($P < 0.001$) and the interaction ($P < 0.001$). Tom recorded the highest mean of 114 days to maturity over the six planting dates and NAV Red the least number of days (102 days).

Days to maturity was least for the 18th June sowing (98.2 days) and greatest for the 1st June sowing (111.3 days) (Table 5)

Total pod yield (kg/ha)

There was a significant difference in planting dates ($P < 0.001$) and landrace ($P = 0.003$) with respect to total pod dry weight. NAV Red produced the highest pod yield of 177.2 g m⁻² (Table 5). There was no significant difference with respect to interaction.

The 19th March planting date produced the highest total pod dry weight of 233.3 g m⁻² relative to the 18th June planting which gave the least mean pod yield of 27.8 g m⁻² for all the landraces (Table 5).

DISCUSSION

Interaction effect

Even though sowing date by landrace interaction effects were observed in some results of this study, Clewer and Scarisbrick (2001; 1991) reported that it is debatable whether different sowing dates can be regarded as factor levels. Little and Hills (1977) also observed that several years' results involving several harvests each year may be combined as a split plot analysis with varieties as the main plots, years as split plots and harvest as split-split plots, however, the interaction of varieties x years x harvests usually is not of primary importance. Main plot effect of this study were therefore discussed even when significant interaction was observed.

Days to 50% emergence and 50% flowering

Sesay (2005) working on time of sowing in Swaziland reported that the longer period from sowing to flowering for the October 13 sowing was due to the delay in seedling emergence caused by the late rains. Differences were observed with respect to planting dates to dates to 50% emergence and days to 50% flowering for the 2008 sowing. The 13/03/08 sowings emerged earlier than the 26/06/08 sowings. Where water is not limiting, temperature may account for differences in seedling emergence and days to flowering. The 23/06/08 sowings were done at the rainy season. Mean temperature from 13/03/08 to 25/03/08 a period of 12 days where emergence is likely to take place, with the sowing day as Day 0 was 33.5°C. The mean temperature from the 26/06/08 to 6/07/08 a period of 12 days with the sowing day considered as Day 0 was 29.1°C. It is noteworthy that March is at the tail end of the dry season in Wench, however, Wench registered a total rainfall of 30.1 mm on the 12/03/08 a day before the 13/03/08 sowing. It is possible that the relatively higher temperature for the March sowing might have resulted in a faster water uptake by the seeds from the stored moisture and hastened seed metabolic activities which could have resulted in quicker seedling emergence.

Pod yield

Pod yields of between 378 kg/ha and 4.6 t/ha were observed in Wench which is in the Transition agro-ecology. A combination of high rainfall, low irradiance due to cloud cover and symptoms of fungal diseases might have resulted in the low pod yields of the sowings done in Kumasi which is in the forest agro-ecology especially in the June planting. These periods fall within the peak of the major rainfall season in Ghana. The yield per plant in Ghana was generally higher in the minor season than the major (Kumaga *et al.*, 2002). It is interesting however, to note that pod yields of 3 t/ha and 2.4 t/ha were registered by Mottled Red and Burkina for the 12th March sowing. It therefore suggests that when bambara groundnut is cultivated at the appropriate time in the forest agro-ecology of Ghana, relatively high yields would be attained.

Pod and seed harvest index: Apart from Tom which registered a low pod harvest index of 0.06, pod harvest indices ranged from 0.54-0.56 for the remaining landraces. Squire (1990) reported harvest index (HI) values of 0.3 to 0.6 for indeterminate crops under wet conditions. Mkandawire and Sibuga (2002) observed pod harvest indices of 0.57 (flat seeded) and 0.58 ridge seeded in a study conducted at Morogoro in Tanzania. Seed harvest index did not necessarily follow the same trend as pod harvest index. Seed harvest index is affected by both the pod yield and shelling percentage which also affect the total seed yield. Even though Ada and Burkina obtained the highest pod harvest index of 0.56 their seed harvest indices of 0.38 and 0.37 respectively were not the highest. This was due to their low shelling percentage of 67 and 66 percent respectively. Mottled Red and NAV 4 with shelling percentage of 74 percent obtained the highest seed harvest index of 0.41. This has implication for breeding not only for a high pod harvest index but also for high shelling percentage.

Vegetative dry weight and 100 seed dry weight

Tom produced the highest mean leaf dry weight (32.8 g/plant) with Ada and Burkina producing the least leaf dry weights (24.8 and 25.7 g/plant) respectively at harvest. Petiole and stem dry weights followed a similar trend. Tom again showed the highest vegetative dry weight. Contrary to the vegetative dry weight, Tom produced the least pod and seed yield, 378 and 276 kg ha⁻¹ respectively in Wenchi. Decreased partitioning to grain competitively favours partitioning towards organs that continue vegetative growth, hence increasing dry matter production and leaf area (Wallace and Yan, 1998). This was observed in Tom which partitioned more dry matter to vegetative organs at the expense of pod formation.

Even though pod yield of Tom was poor, the 100 seed weight was the highest. The poor yield of Tom was therefore manifest in the few number of pods it produced and not the 100 seed weight. Tom is a big-seeded landrace and this attribute could have endeared it to farmers and accounted for their continuous cultivation. Berchie *et al.* (2010) in a survey in three regions of Ghana observed that consumers have a preference for big-seeded bambara groundnut. It is also interesting to note that Tom has a creamish brown colour which is also acceptable by consumers (Berchie *et al.*, 2010).

CONCLUSION

Results of this study showed that time of sowing affected the yield of bambara groundnut in Ghana. Yields were higher in the dry season (February and March) sowings than in the April to June sowings. Bambara groundnut yield was also found to be higher in the Transition than the forest agro-ecology. Pod yields of over 4 t ha⁻¹ were obtained for some of the landraces at some sowing dates.

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Table 1 Days to 50% emergence, 50% flowering, podding and maturity affected by bambara groundnut landraces and sowing dates

Landrace			
Days to 50% emergence	Days to 50% flowering	Days to podding	Days to maturity

Ada	10.8	37.4	66.1	96.5
Black eye	12.7	37.8	67.6	100.9
Burkina	10.9	37.3	66.1	96.4
Mottled Red	11.7	38.5	67.5	101.9
NAV 4	11.2	37.4	67.9	96.9
NAV Red	12.4	38.1	67.1	102.1
Tom	12.1	47.9	77.2	120.0
Mean	11.7	39.2	68.5	102.1
CV%	6.4	3.8	2.6	1.6
LSD (0.05)	0.49	0.98	1.16	1.08
P Value	<0.001	<0.001	<0.001	<0.001

Table 2: 100 pod dry weight, seed dry weight of 100 pods and shelling percentage as affected by bambara groundnut landraces and sowing dates, Wenchi-Ghana.

Landrace	100 pod dry weight (g)	Seed dry weight of 100 pods (g)	Shelling percentage (%)
Ada	80.0	53.3	66.6
Black eye	93.3	68.5	73.4
Burkina	79.4	52.2	65.7
Mottled Red	90.0	66.7	74.1
NAV 4	92.8	68.9	74.2

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NAV Red	96.1	67.2	70.0
Tom	113.9	82.8	72.6
Mean	92.2	65.7	71.0
CV%	15.0	7.9	5.0
LSD (0.05)	9.2	3.4	3.8
P value	<0.001	<0.001	<0.001
Sowing date			
28/02/08	94.8	70.4	74.2
13/03/08	85.7	68.1	79.5
20/03/08	91.4	61.4	67.2
13/04/08	93.3	64.3	68.9
19/06/08	94.8	63.5	67.2
23/06/08	93.3	66.2	71.2
Mean	92.2	65.7	71.0
CV (%)	15.0	7.9	5.0
LSD (0.05)	NS	3.2	2.6
P value		<0.001	<0.001

Table 3: Total pod, shelling percentage and seed yield as affected by bambara groundnut landraces and sowing dates.

Landrace	Total pod yield (kg/ha)	Shelling percentage (%)	Total seed yield kg/ha
Ada	4436	67	2972
Black eye	4631	73	3381
Burkina	4511	66	2977
Mottled Red	4537	74	3357
NAV 4	4508	74	3336
NAV Red	4381	70	3066
Tom	378	73	276
Mean	3914.0	71.0	2779
CV%	39.9	5.0	39.5
LSD	1775	3.82	1260

P value	<0.001	<0.001	<0.05	
Sowing date	28/02/08	3900	74.0	2886.0
	13/03/08	4230	79.0	3341.7
	20/03/08	4373	67.0	2930.0
	13/04/08	4017	69.0	2771.7
	17/06/08	3937	67.0	2637.7
	23/06/08	3013	71.0	2139.2
Mean	3911.7	71.2	2734.3	
CV (%)	12.1	5.0	14.1	
LSD (0.05)	540.0	2.7	483.3	
P value	<0.001	<0.001	<0.05	

Table 4: Total dry weight, pod dry weight, pod harvest index and seed harvest index for bambara groundnut landraces, Wench-Ghana.

	Total dry weight (kg/ha)	Pod dry weight (kg/ha)	Pod harvest index	Seed harvest index
Landrace				
Ada	7925.0	4436	0.56	0.38
Black eye	8505.0	4631	0.54	0.40
Burkina	8095.3	4511	0.56	0.37
Mottled Red	8265.1	4537	0.54	0.41
NAV 4	8219.4	4508	0.55	0.41
NAV Red	8316.2	4381	0.54	0.37
Tom	5861.2	378	0.06	0.05
LSD (0.05)	1032.6	1775.7	0.18	0.06
CV (%)	11.6	39.9	38.4	38.1
Sowing date	28/02/08	3900.0	0.48	0.36
	13/03/08	4230.4	0.50	0.39
	20/03/08	4373.1	0.50	0.33

<i>BAMLINK</i>				
<i>Part B Section B2</i>				
13/04/08	8437.2	4017.2	0.46	0.33
17/06/08	7967.4	3937.0	0.49	0.33
23/06/08	7283.0	3013.3	0.41	0.29
LSD	615.6	540.0	0.04	0.04
CV (%)	6.6	12.1	7.2	10.0

Table 5: Days to 50% emergence, 50% flowering, maturity and pod yield as affected by bambara groundnut landrace and sowing date, CSIR-Crops Research Institute, Kumasi-Ghana.

Landrace				
	Days to 50% emergence	Days to 50% flowering	Pod yield (g m ⁻²)	
Black eye	8.6	44.2	105.2	110.6
Burkina	8.9	44.0	105.8	116.7
Mottled red	8.3	44.4	105.7	149.3
NAV 4	8.7	43.6	106.2	117.2
NAV Red	8.4	42.8	102.2	177.2
Tom	8.6	48.7	114.5	91.7
Mean	8.6	44.4	107.3	127.2
CV (%)	6.6	2.3	3.3	14.2
LSD (0.05)	NS	0.67	2.36	18.0
P value		<0.001	<0.001	0.003
Sowing date				
12/03/08	9.4	44.8	105.7	208.1
19/03/08	8.2	41.5	110.8	233.2
14/04/08	8.3	45.7	111.1	141.6
28/04/08	8.2	41.6	106.6	119.0
01/06/08	8.2	46.1	111.3	33.5
18/06/08	9.2	42.9	98.2	27.9
Mean	8.6	43.8	107.3	127.2
CV (%)	6.6	2.3	3.3	14.2
LSD (0.05)	0.4	0.7	2.4	43.2

<i>BAMLINK</i>			
<i>Part B Section B2</i>			
P value	<0.001	<0.001	<0.001
			0.003

Developing XSpecies Approaches for Genomics and Transcriptomics – Using Resources Developed in Major Species for Research in Bambara Groundnut* Hui Hui Chai, Hung-Ming Lai, Hui Guo, Shravani Basu, Festo Massawe, Sayed Azam-Ali, Neil Graham, Martin Broadley, Sean May and Sean Mayes.

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Keywords: Bambara groundnut, underutilized crop, Affymetrix microarrays, PCR primers, molecular markers

Abstract

Bambara groundnut (*Vigna subterranea* L. Verdc) is a legume mainly grown by subsistence and small-scale farmers in sub-Saharan Africa. This underutilized crop has nutritious and protein-rich seeds, can grow in poor soils and is more tolerant to drought compared to similar major crop species. Like bambara groundnut there are many minor and underutilized crops which have not undergone extensive research yet and for which few genomic resources currently exist. We aim to investigate ways to use resources that have already been developed in major species for crop improvement programs in minor and underutilized species. Affymetrix microarrays are powerful and popular tools for genomic and transcriptomic studies, but they require extensive species-specific sequence information to design. Thus, to save time and reduce cost, one option is to use an XSpecies microarray. Here we have used the XSpecies approach to detect genomic differences between two parents of a bambara groundnut controlled cross between VSSP11 (low stem number per plant) and DipC (high stem number per plant), in order to attempt to develop molecular markers that are linked to the gene(s) controlling stem number as a test case. Bambara groundnut DNA derived from two F₂ bulks that are constructed for high and low stem number, respectively, were hybridized separately to the *Arabidopsis* ATH1 and the *Medicago truncatula* Affymetrix GeneChips. PCR primers were designed from array probe sequences based on hybridization signals visualized in the PIGIONS.v1 software. Differences detected between the two parents and the F₂ bulks *in silico* are being tested in the lab to determine whether this is a useful way to generate markers to traits controlled by limited numbers of genes in underutilized and minor crop species. Results to date will be presented.

INTRODUCTION

Bambara groundnut is an indigenous African legume widely grown in sub-Saharan Africa by subsistence and small-scale farmers. It represents an important source of additional protein to a large proportion of the population in these poor countries. Bambara groundnut is valued for its protein-rich seeds, drought tolerance, adaptability to poor soils and resistance to pests and diseases. It consists of two botanical forms: var. *spontanea* comprising the wild forms, restricted to Cameroon and var. *subterranea* comprising domesticated forms, which can be found in most of the tropical areas, especially in sub-Saharan Africa. As there are no established varieties, most of the marginal and subsistence farmers in Africa grow locally adapted landraces which can result in poor and/or unpredictable yields. A cross was made between wild (VSSP1; 'spreading') and domesticated (DipC; 'bunched') bambara groundnut landraces. The F₁ hybrid was self-pollinated and the resultant F₂ population was used to study a range of agronomic and domestication traits, including days to emergence, days to flowering, internode length at harvest and number of stem per plant number as reported in Basu *et al.*, 2007. The authors suggested that a possible 1:2:1 segregation pattern is observed in the F₂ population, consistent with a χ^2 test ($P=0.03<5.99$; 2 df) for the stem number trait, indicating that phenotypic variation in the cross for this trait is controlled by single locus. Together with the trait 'stem length', these two traits account for the majority of the

PIGEONS Software was used to analyze the CEL files that resulted from the XSpecies experiments in order to generate a candidate list for potential probe sets that gave high signal strength as well as those that showed differences in signal between VSSP11 and DipC. The two parents VSSP11 and

XSpecies Analysis
DNA extracted from bambara groundnut were sent to the NASC Affymetrix chip service, Plant and Crop Sciences, Sutton Bonington, for cross hybridization with Affymetrix *Arabidopsis* ATH1 and *Medicago truncatula* GeneChips, respectively.

Genomic DNA Extraction
Genomic DNA was extracted from bambara groundnut leaf tissues using the GenElute Plant Genomic DNA Miniprep Kit (Sigma, UK). The concentration of DNA (ng/μl) was estimated using the Nanodrop 1000 (Thermo Scientific, USA) associated with ND-1000 V 3.7.0 software.

Plant Materials
Two parents of a bambara groundnut controlled cross between VSSP11 (*V. subterranea* var. *spontanea*) from Cameroon and DipC (*V. subterranea* var. *subterranea*) from western Botswana as well as an F₂ population which was subsequently constructed into DNA bulks for high and low stem number were used in this study.

MATERIALS AND METHODS

In this paper, we aim to use the XSpecies approach to detect genomic differences between two bambara groundnut accessions (VSSP11 and DipC) in order to try to develop molecular markers that are linked to the gene(s) controlling stem number. The genomic resources from model plant, *Arabidopsis*, and model legume species, *Medicago*, will be adopted for research and breeding work in bambara groundnut.

Sixteen Affymetrix microarrays are now available for plant species (Affymetrix, 2011) and more are coming in the near future. Microarrays have become a powerful and popular tool for genomic and transcriptomic studies, however it requires extensive sequence and genome information to design. XSpecies microarrays have emerged as they allow the identification of oligonucleotide targets of one species by hybridizing nucleic acids onto the Affymetrix microarray derived from another species which is currently available, such as *Arabidopsis*, soybean or *Medicago*. The XSpecies approach is a combination of physical and bioinformatics methods, with an appropriate analysis after the hybridization required in order to generate a valid result. A program which is known as 'Photographically Integrated En-suite for the OligoNucleotides Screen' (PIGEONS), was developed and used to investigate the individual oligonucleotides underlying genomic cross-species studies (Lai, 2009). There are many minor and underutilized crops like bambara groundnut that have not undergone the extensive research yet needed to generate dedicated microarrays. XSpecies microarray hence provides an alternative option for crop improvement programs in minor and underutilized crops through the use of genomic resources that have already been developed in major crops.

This paper reports results to date from the XSpecies approach. Parallel Signature Sequencing have been investigated for the genetic improvement of bambara groundnut. Nearly one hundred SSR markers have been developed for fingerprinting, diversity analysis and mapping in bambara groundnut. DART microarray technology, Affymetrix XSpecies microarrays, QTL analysis of a 'wide' and 'narrow' crosses and the development of Massively Parallel Signature Sequencing have been investigated for the genetic improvement of bambara groundnut. This paper reports results to date from the XSpecies approach.

Molecular approaches have been widely used for the genetic improvement of crop simple traits and to develop potential markers for selection.

for testing when molecular approaches can be applied to further investigate the genetic basis of such morphological difference between the wild and domesticated parents. Thus it is a good candidate

DipC were selected as 'parent1' and 'parent2' while F₂ offspring constructed into bulks for 'high' and 'low' stems per plant were selected for analysis.

RESULTS AND DISCUSSION

Threshold Selection

The threshold boundary for the XSpecies analysis result from the hybridization of bambara groundnut DNA on Affymetrix ATH1 GeneChip is between 80 to 160. A Threshold value of 120 was chosen as it is in the middle of the suggested threshold boundaries, with a relatively high probe set retention rate, 93.75%, and 3.1 for the ratio of average probe pairs to probe sets (Fig. 1). For the results of the hybridization on the Affymetrix *Medicago truncatula* GeneChip, the threshold value of 100, with probe set retention rate 88.28% and ratio of average probe pairs to probe sets, which is 3.2, was selected (not shown). The Medicago chip also represents a number of genes derived from non-Medicago species, such as nodulation bacteria, which may account for the lower retention rates, despite *Medicago truncatula* and bambara groundnut both being legumes. Threshold boundaries are useful for giving an idea of which threshold should be chosen for analysis, as well as the number of probe pairs, probe sets and ratio of average probe sets to probe pairs. It is suggested that when the threshold is increased from 0 to 1000, probe pairs are lost rapidly although entire probe sets which represent transcripts are lost relatively slow as only a minimum of one PM probe is required to retain a probe set (Hammond et al., 2005). It is essential to remove bad probe sets and/or probe pairs which have low threshold value to increase the specificity of cross-hybridization.

Potential Probe Set Identification

The potential probe sets are now analysed in two stages. Firstly, at the defined threshold value, with fold change set as 2 for 'P' and 1.5 for 'F₂', potential probe sets which have high value of PM signals from Affymetrix array as well as large differences in signal between the two parents or the two F₂ bulks are identified. The screen result is shown in Fig. 2, using data from cross hybridization of bambara groundnut on Affymetrix *Medicago truncatula* GeneChip as an example. The figure shows that the probe set, Mtr. 25972.1.S1_{at}, obtain PM signals that are above 1000 as well as high signal difference between the parents and the F₂ bulks. Secondly, an effort will be made to design PCR primers from array probe sequences on the basis of hybridization signals that have been observed in PIGEONS software. *In silico* detection of the differences between the two parents and the F₂ bulks will be tested in the lab to determine whether the polymorphism is either genuine or due to background noise. Good candidates will show strong amplification and hopefully polymorphism in the region where a signal difference was detected in PIGEONS. Such differences can then be used to map putative markers for stem number, as illustrated in Fig. 3, using an example of primers designed from 262850_{at} which is suspected to be the GAI gene in *Arabidopsis*.

Background noise is one of the major problems when XSpecies approach is used for molecular work in bambara groundnut. Noises can result from the inefficient hybridization of certain transcripts to the probes on the array due to sequence polymorphisms between two different species (Hammond et al., 2005). To reduce noise, several approaches could be adopted. One of them is to increase the number of individual plants within the bulks constructed for low stem and high stem number per plant. The original sequences of probe sets can be obtained from Affymetrix website. Sequences can then be screened via TblastX with databases and sequences that are currently available to look for sequences that align with bambara groundnut. Primers are designed using aligned sequences from bambara groundnut for more specific PCR amplification. For species that are separated by large genetic distances, only the more conserved genes may be directly comparable.

CONCLUSION

Using resources developed in major crop species for research in minor and underutilized crop species is one way to develop genomic resources in species where sequence information is still

limited. The recent introduction of high-throughput DNA sequencing instruments, so-called "Deep Sequencing" technologies for "next-generation sequencing" have the potential to identify polymorphisms between individuals, for gene expression analysis and mutation discovery (Mardis, 2008). Deep Sequencing can be used to produce extensive sequences which will allow the development of markers as well. These approaches are comparatively inexpensive and allow the production of millions of DNA sequence reads per instrument run. It is thus suggested that the combination of the use of deep sequencing and XSpecies may accelerate the generation of markers to traits controlled by limited numbers of genes in underutilized and minor crop species.

BAMLINK

Part B Section B2

ACKNOWLEDGEMENTS

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Figures

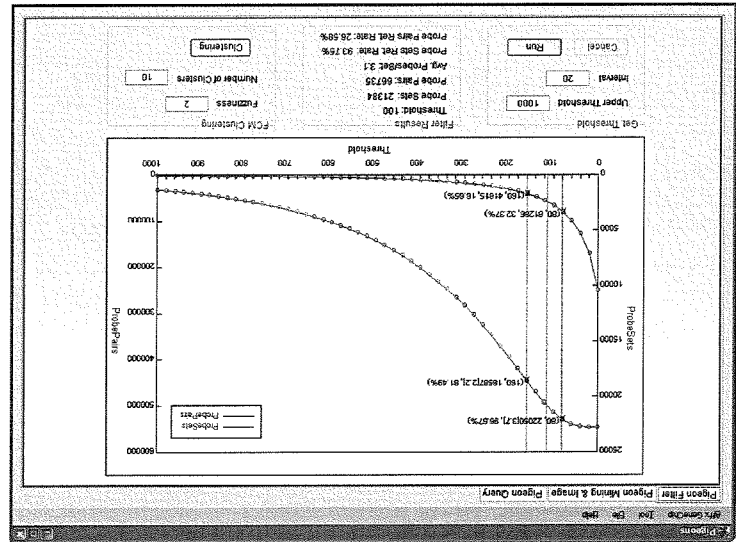


Fig. 1. Threshold boundaries for the XSpecies analysis result from the hybridization of bambara groundnut DNA on Affymetrix *Arabidopsis* ATH1 GeneChip.

Fig. 3. Primer designed from probe set 262850_at is used to amplify bambara groundnut DNA. L: 1kb DNA ladder; D: DipC; V: VSSP11; A: *Arachidopsis* (positive control).

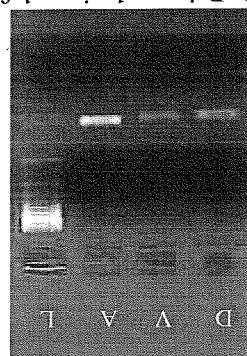
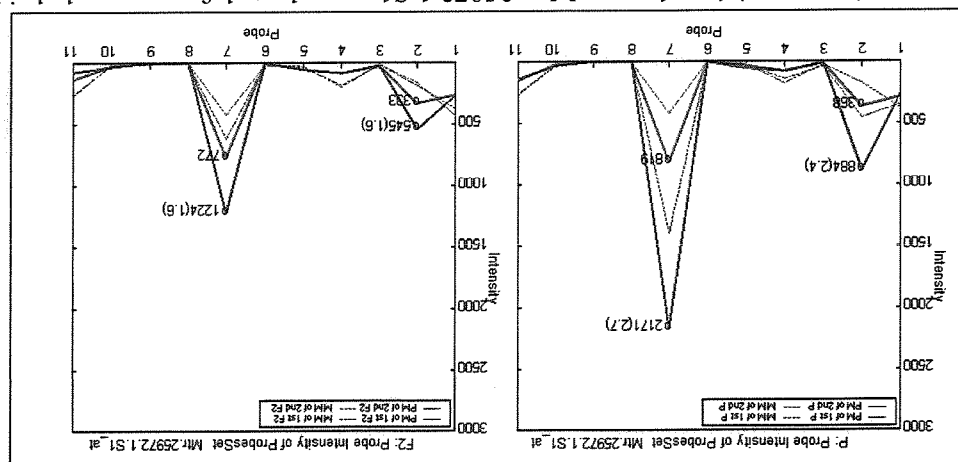


Fig. 2. The potential probe set, Mtr. 25972.1.S1_at, selected from cross hybridization of bambara groundnut on Affymetrix *Medicago truncatula* GeneChip.



-Stability and adaptability of bambara groundnut landraces Redjeki, E. S.^{1*}, Mayes, S.², Azam-ali, S.³

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Keywords: stability, adaptability, regression coefficient, deviation from regression

Bambara groundnut has been planted in Indonesia for hundreds of years. Researchers have evaluated where the Indonesia bambara groundnut landraces were introduced from but none has evaluated the stability and adaptability of bambara groundnut in Indonesia. Thirty-six landraces were planted in Indonesia, together with putative Indonesian x African hybrids and their offspring. These were assessed for their stability and adaptability by the methods of Finlay and Wilkinson (1963) and Eberhart and Russel (1985). Results from seven landraces are presented. The seven landraces were: 'Lunt' from Sierra Leone, 'AHM753' from Namibia, 'SB165A' from Namibia, 'DODR' from Tanzania, 'Uniswa Red' from Swaziland, 'DIPC' from Botswana and 'S19-3' from Namibia, with the Indonesian landrace 'Gresik' as control. Thirty plants of each landrace were planted in a randomized block design with three replicates at Gresik, Bojonegoro and Jatikerto in Indonesia in November 2009. Each location had a different altitude, soil type and rainfall. Gresik is the main bambara groundnut growing region in the East of Java, Indonesia. Prior to this experiment, farmers in Bojonegoro and Jatikerto were not familiar with this crop. Many traits were assessed based on the list of descriptors of bambara groundnut issued by IPGRI, but in this report we present only the results of stability and adaptability analysis for 50% flowering, days to maturity, pod number per plant and the 100 seeds weight traits. Analysis of variance showed highly significant differences in all three locations and further combined analysis of variance over sites (Gomez and Gomez, 1983) indicated that location, landraces, interaction between location and landraces revealed highly significant differences. Stability and adaptability parameters were obtained as the linear regression coefficient (bi) of the mean of all data observed and deviation from the regression analysis (S^2_{di}) with the hypothesis that $b_i = 1$ and $S^2_{di} = 0$. The results indicated almost all landraces observed were stable, but only two landraces have good adaptability in all three locations, namely 'SB165A' and 'Uniswa Red'. Lunt, DIPC and S19-3 are considered as promising landraces because they are well adaptable in two of the four variables used. This information could be useful for further breeding programs.

INTRODUCTION

Bambara groundnut is not a native crop of Indonesia. Records suggest that it came from South-West Africa in the early of 18th century through trade links. The crop has numerous colours of seed testa, namely; brown, cream, red and often has variegated testa. Due to farmers selectively planting certain colours, the colour of seed testa planted in Indonesia today is largely black, dark red, dark brown and dark purple. Indonesian farmers prefer to plant seed having dark coloured testa and a white hilum. Nutritional content of this crop is comparable to other legumes. Linnemann and Azam-ali (1993) estimated that the protein content of bambara groundnut was between 16-25%. This legume crop is suitable for semi-arid climates, is relatively resistant to diseases and pests (Linnemann and Azam-ali, 1993) and has the potential to generate high yields (Collinson et al., 2000). Even though this crop is broadly distributed in Africa and South East Asia, until recently bambara groundnut did not receive appreciable research effort, particularly for genetic improvement (Massawe et al., 2005) so that bambara groundnut exists as landraces rather than varieties, which

MATERIAL AND METHODS

significantly complicates genetic analysis and breeding. However, it may assist crop survival under harsh environmental conditions (Zeven, 1998). Most researchers who work on bambara groundnut (Karikari, 2000; Basu *et al.*, 2007; Mwale *et al.*, 2007; Ntundu *et al.*, 2006) considered that bambara groundnut has many useful traits for crop improvement.

In a study of 27 genotypes under optimal agronomic conditions in Ghana (Karikari, 1972), a simple correlation analysis indicated that number of stems per plant and 100 seed weight were positively correlated with grain yield and these characters were used for selection. The characterisation and evaluation of bambara groundnut at the International Institute of Tropical Agriculture (IITA) revealed enormous agro-morphological diversity which could be used for crop improvement. In a correlation matrix study of the IITA collection, Goli *et al.* (1997) found that the characters most strongly correlated with grain yield were number of leaves, pods, shell thickness and 100 seeds weight. Karikari (2000) stated that 100 seed weight was found to be the most important character to be considered during selection and breeding of bambara groundnut in Botswana and areas with similar climates. Masindeni (2006) also revealed that the seed number per pod and 100 seed weight had highly positive correlations to grain yield. Meanwhile Misanu *et al.* (2007) observed that there was a negative correlation between 100 seed weight and pod number of bambara groundnut in a screen house experiment in Tanzania. They also suggested that early flowering of landraces could represent an advantage in terms of forming pods for a longer period of time to generate more pods, while late flowering resulted in a decrease in the seed yield. Another experiment in Nigeria (Jonah *et al.*, 2010) found a positive genotypic correlation between seed and pod number per plant. Makanda *et al.*, (2009) considered that pod number per plant should be an important trait in the further development of bambara landraces.

Some landraces may have good performance in a specific environments, but poor in several others. The ability of some crops to perform well phenotypically across a wide range of environmental conditions has been examined by plant breeders and agronomists. Allard and Bradshaw (1964) considered that the stability of landraces can reflect its adaptability to environment changes. A landrace can be stable to environments if it gives limited deviation in response to different environments (Becker and Leon, 1988). Finlay and Wilkinson (1963) characterized the stability of landraces based on a regression coefficient (b_i) with the following classification: $b_i = 0$, absolute stability; $b_i < 1$, average stability; $b_i > 1$, above average stability and $b_i > 1$, below average stability. Moreover Eberhart and Russel (1966) examined adaptability which correlated b_i and mean yield. A landrace which has regression coefficient (b_i) = 1 will be said to be adaptable in general. However when general adaptability is correlated to a high mean yield, it can be said to be well adaptable. Moreover it can be said to be poorly adapted when correlated to a low mean yield. A landrace which has above average mean yield in an unfavourable environment, but is low yielding in a good environment can be said to have a specific adaptability to that unfavourable environment and vice-versa for a landrace with a specific adaptability to favourable environment.

The current problem of bambara groundnut in Indonesia is that it has a long life span and is low yielding (< 1 ton/ha²). Even though it has a high market price, farmers are reluctant to plant it because of the time to maturity. However a new variety which has desirable traits - particularly a high yield and quick maturing - is needed (although it may be difficult to achieve both at the same time). Several field experiments were conducted in Indonesia to assess the desirable traits of bambara groundnut for development of a potential new variety.

The aims and objectives of this project are to evaluate bambara groundnut landraces in Indonesia environment. Selected bambara groundnut landraces were planted and measured as single plants to examine their stability and adaptability in three different locations. Hypotheses: There are differences in stability and adaptability of bambara groundnut landraces based on the coefficient of regression ($b_i = 1$), deviation from regression slope ($S^2_{di} = 0$) and grand mean of variable measured.

This experiment was conducted during the rainy season from November 2009 to May 2010 in three locations including: Gresik, Bojonegoro and Jember in Indonesia. The detailed sites of the experiments are listed in Table 1. The material used was from the seed lots which are available in the seed store of Tropical Crop Research Unit, School of Biosciences of The University of Nottingham, United Kingdom. The landraces used and their original country are provided on Table 2.

A Randomized Block Design with eight treatments (landraces) and three replications in each location was used. Randomization of landraces was done for each block, which has area of 2 x 1.5 m² per plot. The seeds were planted at a spacing of 40 cm between rows with spacing of 25 cm within rows. A single seed was planted in each hole at 5 cm in depth so that each landrace had 30 single-plants. Any replanting for non-emergent seed was done two weeks after sowing. Each block was separated by drainage channels (50 cm between replication and 30 cm in depth) to keep the plants growing well during the rainy season. De-weeding was done frequently due to high competition between the plants and the weeds in rainy season. Data for many traits were collected, such as growth and yield variables, but in this paper we report only four illustrative traits including: days to 50% flowering, date of maturity, number of pods per plant, and 100 seed weight. Fifty percent flowering is the number of days from sowing to when 50% of the plants in one plot had started flowering. Data was collected every day starting from when the first flower appeared until at least 15 plants (50%) per plot have flowered. Date of maturity was calculated based on the time from sowing to the harvesting (days after sowing = DAS). Each plant was harvested when the leaves were yellow and dry; pods had hardened and were white in colour. Pod number per plant represented the number of pods per plant grown in the field. It was measured at harvest time for the mature pods only. One hundred seeds weight was counted based on the weight of 100 seed with three replicates per plot.

Stability and adaptability analysis

Stability and adaptability can be analysed by linear regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968; Freeman and Perkin, 1971). Analysis of variance in stability analysis was conducted based on linear regression equation from Eberhart dan Russel (1966) and Roy (2000) generated from many researchers such as:

$$Y_{ij} = m_i + \beta_i I_j + \delta_{ij} \quad (i = 1, 2, 3, \dots, t \text{ and } j = 1, 2, 3, \dots, s) \rightarrow t=8 \text{ and } s=3$$

Y_{ij} = mean data for i- landrace in j- location
 m_i = grand mean
 β_i = regression coefficient of i- landrace
 I_j (Location index) = $\frac{\sum_i Y_{ij}}{\sum_i Y_{..}} - \frac{t}{ts}$

δ_{ij} = deviation from regression for i-landrace and j-location
Regression coefficient (b_i) and deviation from regression (s^2_{di}) can be estimated as:

$$b_i = \frac{\sum_j Y_{ij} I_j}{\sum_j I_j^2}$$

where: $\sum_j Y_{ij}$ = sum of mean data for i- landrace in j- location
 $\sum_j I_j^2$ = sum square of location index

$$\text{Deviation from regression} = S^2_{di} \rightarrow S^2_{di} = \frac{\sum_j \delta_{ij}^2}{s-2} - \frac{S^2_e}{r} \text{ where:}$$

$$\sum_j \delta_{ij}^2 = [\sum_j Y_{ij}^2 - (\sum_j Y_{ij} I_j) / (\sum_j I_j^2)]^2$$

S^2_e = Pooled Error estimated

s is number of locations and r is number of replicates. For further analysis we test for significance of the sources of variance with F-test 5 % and 1 %.

Data interpretation:

As explained previously stability and adaptability analysis are based on the Finlay and Wilkinson (1963) and Eberhart and Russel (1968) methods, we can conclude that:

1. When the approximated regression coefficient is nearly 1 it means that a landrace has average stability. An average stability landrace can have a general adaptability if the landrace mean is greater than grand mean of landraces overall in that environment. However when an average stability of landrace is associated with low mean of the variable measured, that landrace is considered to be poorly adapted to all environments.
2. A regression coefficient more than 1 ($b_i > 1$) means that a landrace has a below average stability and is more sensitive to environment changes. It can be said to have specific-adaptability in a good environment.
3. A regression coefficient below 1 ($b_i < 1$) suggests a landrace has above average stability and with more sensitivity to low-yielding environments.

RESULT AND DISCUSSION

Variables in this experiment included '50% flowering', 'days to maturity' (from days after sowing; DAS), 'pod number per plant' and '100 seeds weight' (g). Mean squares of analysis of variances and coefficient of variance (%) are listed in Table 3. Landraces showed highly significantly differences for all yield variables used. Meanwhile there is no significant difference between blocks (replication) in the three individual locations (Gresik, Bojonegoro and Jatikerto). Further analysis of the mean of yield variables was done by LSD 5% (Gomez and Gomez, 1983). Table 4 lists the mean of eight landraces in three locations for '50% flowering' (DAS), 'days to maturity' (DAS), 'pod number per plant' and '100 seeds weight', respectively. Seven African landraces showed different responses. In the Gresik field Lunt, SB165A and DODR reaches 50% flowering faster than the Gresik landrace used as control. Meanwhile in the Bojonegoro field, Lunt, DODR and S19-3 showed the earliest time for '50% flowering' again compare to Gresik landraces. In the Jatikerto field, almost all African landraces planted reached '50% flowering' earlier than the Gresik landrace (Table 4). Days to maturity for eight bambara groundnut landraces planted in the Gresik field were almost the same, except AHM753 from Namibia. It had 123.7 DAS of date maturity and is quicker than other landraces used. Surprisingly, AHM753 in the Bojonegoro field has the same date of maturity as the Gresik landraces DIPC and Uniswa Red, which are nearly 121.75 DAS. The earlier ones are Lunt, SB165A, DODR and S19-3. The Gresik landrace is still the longest one for days to maturity in three locations. The pod number per plant in three locations showed various responses. AHM753 is promising in the Gresik and Bojonegoro fields. Meanwhile S19-3 has the greatest number of pods per plant in the Jatikerto field. SB165A and Gresik landraces tend to have the same weight of 100 seeds in Gresik and Bojonegoro. Meanwhile in Jatikerto, SB165A and DODR are the heaviest ones.

Landraces (G) and environment (E) interaction

Analysis of variance over sites is listed in Table 5. Interaction between landraces and locations were highly significant ($P < 0.01$) for 50% flowering, days to maturity, pods number per plant and 100 seeds weight. That means we can find at least one landrace which is suitable for a particular location or suitable for all locations used. The mean square of all variables used for data interaction analysis is displayed in Table 6.

Stability and adaptability analysis

Further analysis to observe stability and adaptability of landraces was conducted based on Finlay and Wilkinson (1963) and Eberhart and Russel (1966) analysis. A landrace will be estimated stable if it has deviation from regression (S^2_{di}) = 0; regression coefficient (b_i) = 1 and mean of the variable for that landrace is greater than its grand mean. Original data was transformed to a logarithmic scale (Finlay and Wilkinson, 1963). Analysis of variance in stability analysis for 50% flowering, date of maturing, pod number per plant and 100 seeds dry weight are displayed from Table 7.

In this study, the Gresik landrace control showed poor adaptation in 50% flowering due to having the longest time for flowering and was unstable in three other variables including, days to maturity, pod number and 100 seed weight.

Indonesia has various environments which are suitable for crops. Gresik, Bojonegoro and Jatikerto are agricultural area. Gresik is the centre of bambara groundnut in Indonesia even though only a few Indonesian farmers know about it. Meanwhile Bojonegoro and Jatikerto are new locations for bambara groundnut due to farmers being unfamiliar with this legume crop.

CONCLUSION

Result of stability and adaptability analysis suggested that three of seven African landraces namely: SB165A and Uniswa Red are well adapted in Gresik, Bojonegoro and Jatikerto. Meanwhile Lunt and DIPC are considered as promising landraces because of being well adapted in two of four variables used. This information could be useful for further breeding program.

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Table1. Details of site of the experiment

Differences	Site of Experiments		Gresik		Bojonegoro	Jatikerjo
	Soil type					
	Sandy (%)	17	53	35		
	Loam (%)	54	32	27		
	Clay (%)	29	15	38		
	Average rain fall per month (mm)	81	295.2	360.75		
	Altitude (m)	5	18	335		
	pH	6.0-6.7	6.8-7.2	5.4-6.2		
	Temperature Min-Max (°C)	22-35	22-35	21-33		

Table2. Landraces used and country of origin

No	Landraces	Original Country
1	Lunt	Sierra Leone
2	AHM753	Namibia
3	SB165A	Namibia
4	DODR	Tanzania
5	Uniswa Red	Swaziland
6	DIPC	Botswana
7	S19-3	Namibia
8	Gresik	Indonesia

Table 3. Mean Square of analysis variance and CV (%) for 50% flowering, date of maturity, pods per plant and 100 seeds in three locations

Location	Source	df	50% flowering (das)	date of maturity (das)	Pod number per plant	100 seeds weight(g)
Gresik	Landraces	7	13.23**	65.90**	897.18**	355.81**
	Block	2	14.00	6.50	0.63	43.94
	Error	14	1.29	13.12	1.48	29.54
	CV (%)		2.47	2.70	2.49	12.07
Bojonegoro	Landraces	7	4.48**	207.02**	232.52**	117.65**
	Block	2	0.29	120.79	4.96	42.31
	Error	14	0.82	38.51	1.76	10.62
	CV (%)		2.04	5.40	7.38	14.03
Jatikerto	Landraces	7	20.23**	218.04**	143.78**	192.03**
	Block	2	1.63	55.13	1.93	51.75
	Error	14	0.63	68.51	1.46	34.61
	CV (%)		1.87	5.46	4.13	12.75

Table 4. Mean of eight landraces in three locations for '50% flowering', 'days to maturity', 'pod number per plant' and '100 seeds weight'

Landraces	50% flowering (DAS)		days to maturity (DAS)		pod number per plant		100 seeds weight (g)	
	Gresik	Bojonegoro	Gresik	Bojonegoro	Jatikerto	Gresik	Bojonegoro	Jatikerto
Lint	43.00 a	42.33 a	41.33 ab	137.67 b	105.00 a	140.00 a	25.96 a	12.79 b
AHM753	43.33 b	43.67 bc	41.33 ab	123.67 a	117.67 bcd	150.33 ab	83.92 f	23.64 e
SB165A	47.33 a	43.33 cd	40.33 a	137.00 b	114.00 abc	156.33 bc	50.91 d	15.26 c
DODR	44.00 a	44.00 abc	41.33 ab	135.67 b	105.00 a	146.67 ab	42.46 c	16.21 a
Untwa Red	47.33 b	44.33 cd	42.00 b	136.67 b	118.67 bcd	157.33 bc	48.96 d	18.14 d
DIPC	47.33 b	45.00 bc	44.33 c	136.67 b	124.00 cd	153.00 abc	43.42 c	20.10 d
SI9-3	46.33 b	44.67 ab	40.33 a	131.33 b	108.33 ab	143.00 ab	59.21 e	12.53 b
Gresik	48.33 b	46.33 d	48.00 d	133.33 b	126.67 d	166.33 c	36.29 b	35.35 f
LSD5%(df=14)=2.145	1.99	1.58	1.88	6.34	10.87	14.50	2.13	2.32
Mean	45.14	44.14	44.14	132.14	118.14	153.14	50.14	17.14

Note: Mean which is followed the same alphabet indicated no significant difference at LSD5%

Table 5. Analysis of variance over sites for '50% flowering', 'days to maturity', 'pod number per plant' and '100 seeds weight'

Source of variation	Degree of Freedom	50% flowering	date of maturing	Pod number per plant	100 seeds weight
Location (E)	s-1	73.85**	8089.27**	5872.74**	4005.39**
Replication within location	(r-1)s	6	5.31	60.81	46.00
Landraces (G)	t-1	7	25.96**	274.70**	547.38**
G X E	(t-1)(s-1)	14	5.99**	108.14**	363.06**
Pooled Error	sr-1(r-1)	42	0.91	40.04	1.57
Total	rst-1	71			24.92

Note:**indicated highly significance at F-test 1%

Table 6. Mean square of analysis of variance interaction

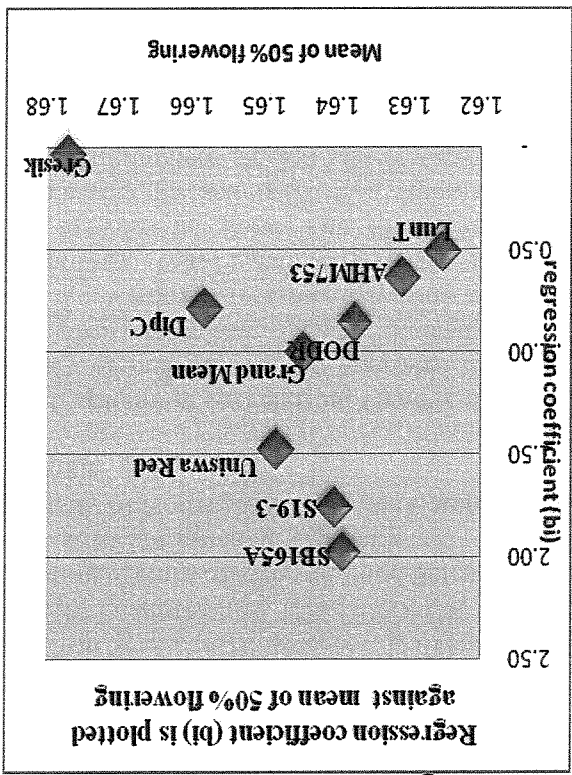
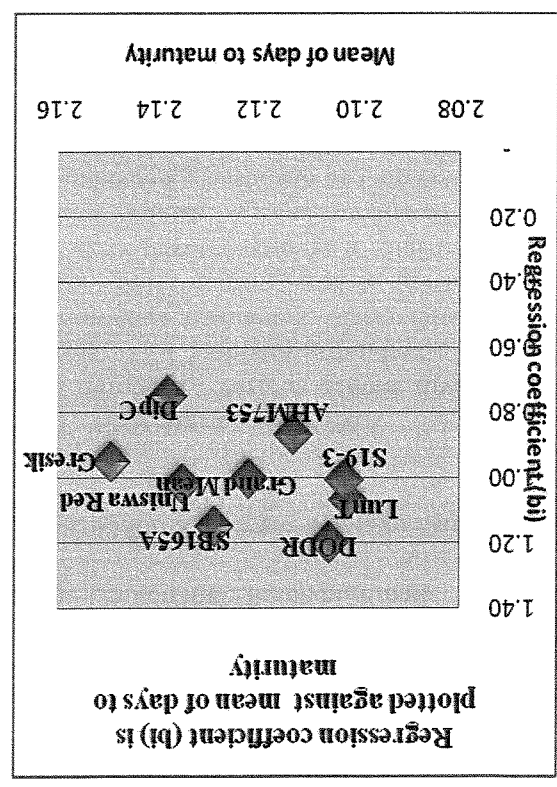
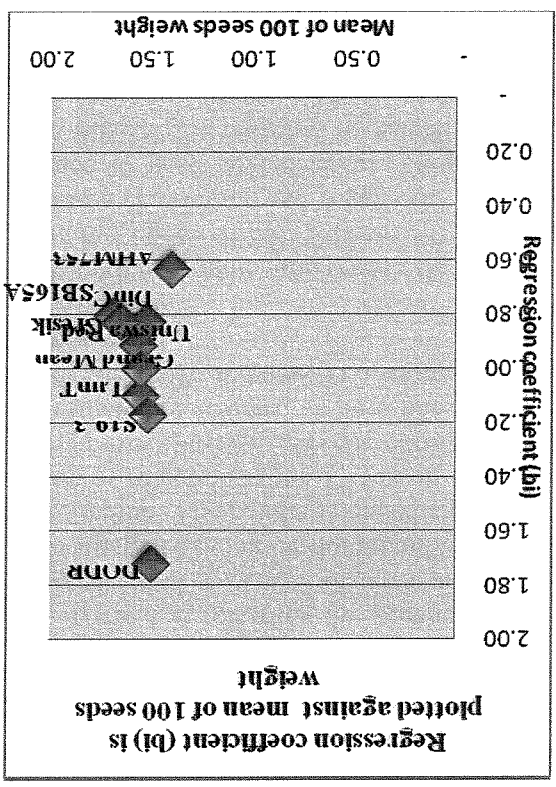
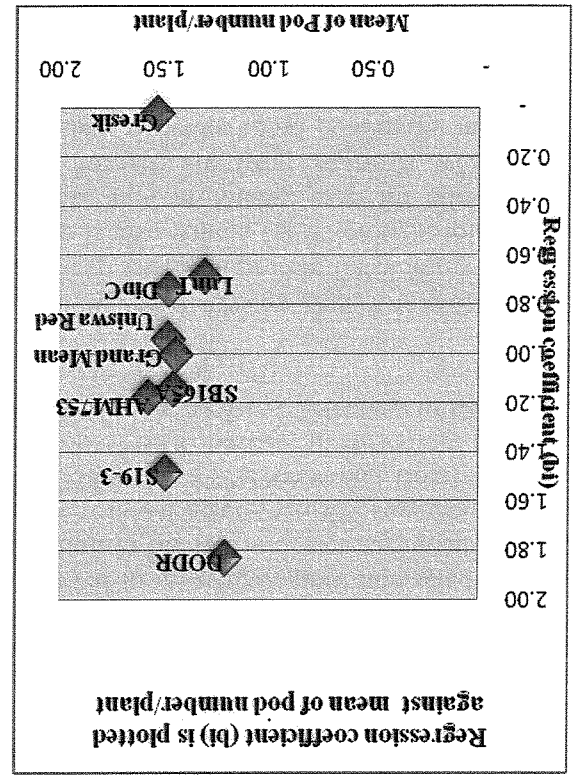
Source	df	50% flowering (DAS)	days to maturity (DAS)	Pod number per plant	100 seeds dry weight (g)
Landraces	7	0.0025960**	0.00300**	0.13806**	0.07395**
Location	2	0.00738**	0.08930**	1.27150**	0.72275**
Landraces*Location	14	0.00060**	0.00136**	0.06153**	0.01381**
Error	48	0.00015	0.00048	0.00065	0.00388

das = days after sowing
** and * indicated significant difference at F-test 1% and 5%, respectively

Table 7. Deviation from regression (S^2_d) and regression coefficient (b) for '50% flowering', 'days to maturity', 'pod number per plant' and '100 seeds weight'

No	Landraces	50% flowering		Days to maturity		Pod Number/plant		100 seeds weight	
		S ² d	F _{calc}	b _i	F _{calc}	b _i	F _{calc}	b _i	F _{calc}
1	Lint	-0.0005	0.00019	0.5060	1.3215	1.62544	0.00134**	0.5459	1.5529
2	AHM753	0.0005	1.95506	0.6311	0.90455	1.63104	0.00079*	0.6352	2.0196
3	SB165A	0.00001	1.14603	1.97183	2.39599	1.63910	-0.00015	0.80588	1.68878
4	DODR	0.00025*	6.5964	0.85221	0.36436	1.63757	0.00049*	0.39479	1.49756
5	Untwa Red	-0.00002	0.59662	1.47096	1.16112	1.64828	-0.00013	0.91157	1.59754
6	DIPC	0.00002	1.34571	0.78491	0.53025	1.65822	-0.00011	0.82809	0.94740
7	SI9-3	0.00001	1.24087	1.75451	1.86020	1.64030	0.00001	1.17207	1.51141
8	Gresik	0.00015	4.04174	0.03187	2.38685	1.67707	0.00117**	0.82683	0.95434
F _{5%} (1,42)=4.07 F _{1%} (1,42)=7.28 F _{5%} (6)=2.45									
** and * indicated significant difference at F-test 1% and 5%, respectively									
9	Gresik	0.00180**	68.3081	1.8041	1.73538	1.49264	0.00044*	4.43156	1.64895
10	Untwa Red	-0.00002	0.59662	1.47096	1.16112	1.64828	-0.00013	0.91157	1.59754
11	DIPC	0.00002	1.34571	0.78491	0.53025	1.65822	-0.00011	0.82809	0.94740
12	SI9-3	0.00001	1.24087	1.75451	1.86020	1.64030	0.00001	1.17207	1.51141
13	Gresik	0.00180**	68.3081	1.8041	1.73538	1.49264	0.00044*	4.43156	1.64895

BAMLINK
Figure 1. Regression Coefficient (b_i) are plotted againsts mean of '50% flowering', 'days to maturity', 'pod number per plant' and '100 seeds weight'
Part B Section B2



Assessment of Genetic Variability of Bambara groundnut (*Vigna subterranea* (L.) Verdc) Accessions using Morphological traits and Molecular Markers O. Molosiwa¹, S. Basu¹, F. Stadler², S. Azam-Ali³, and S. Mayes¹

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Keywords: Cophenetic correlation, Genetic distance, DART, SSR marker, UPGMA

The efficiency of breeding can be improved by employing a number of various genetic diversity measures of crop germplasm. Genetic diversity and relatedness of 24 bambara groundnut (*Vigna subterranea* L. Verdc) was estimated using the (36) morphological characters, (201) Diversity Array Technology (DART) and (65) simple sequence repeats (SSR) markers and to assess the relationship between the three. The 24 bambara groundnut were selected among a total of 119 bambara groundnut accessions (87 from International Institute of Tropical Agriculture and 37 from University of Nottingham stocks) which were planted in an unheated glasshouse at University of Nottingham in May, 2008. Data on the variation for morphological and agronomic traits were recorded following the IPGRI descriptors (IITA, BAMNET (2000). Samples were also analysed with microsatellites markers which were characterised in this project. In addition, DNA extracted was sent for DART analysis.

The study has demonstrated the utility of the three markers in genetic diversity and relatedness. The cophenetic coefficient obtained by the Mantel tests to compare the different markers systems showed that all UPGMA clusters had a good fit to the similarity estimates (Morphological markers $r = 0.82$, DART $r = 0.96$, and SSR $r = 0.83$). The markers provided consistent information as they produced relatively similar pattern of clusters, but DART markers produced a more robust cluster which grouped landraces based on their areas of origin. However, it was the SSR markers which were more efficient in revealing the genetic variability of the 24 bambara groundnut landraces, by recording larger average genetic distance and range based on Nei's genetic distance. SSR markers with the advantage of being co-dominant have been employed to investigate pure line selection of bambara groundnut. The results revealed significant reduction in the levels of heterozygosity from the 1st season of selection to the 3rd season with no signs of residual heterozygosity suggesting that these lines (81-Acc-385TZA, 84-Acc 696ZMB, 88-AHM 753NAM, S19-3NAM and Bots1-BWA) have been effectively selected as pure lines. The application of SSR markers in this study further demonstrates their effectiveness as a technique suitable for Marker Assisted Selection in bambara groundnut breeding.

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an indigenous African crop, it is grown and consumed in most parts of Sub-Saharan Africa and it is a cheap source of protein for majority of the rural population (Linneman and Azam-Ali, 1993). The crop is also valued for its drought tolerance and resistance to pests and diseases. Despite its potential as a promising crop for alleviating poverty and hunger, the crop has no established varieties. Farmers are using landraces, which are usually low yielding (Zeven, 1998). The development of new varieties could potentially increase the yields of bambara groundnut.

The efficiency of breeding can be improved by employing various genetic diversity measures of crop germplasm, such as the use of morphological, biochemical and molecular markers. Morphological markers are the standard and usually the first step in germplasm characterisation, especially in underutilised crop species such as bambara groundnut (Azam-Ali *et al.*, 2001). A number of authors (Goli *et al.*, 1995; Karikari, 2004; Ntundu *et al.*, 2006 and Quedraogo *et al.*,

Plant materials and DNA extraction: A total of 119 Bambara groundnut accessions (87 from International Institute of Tropical Agriculture and 37 from University of Nottingham stocks) were planted in an unheated glasshouse at the University of Nottingham in May, 2008. A subset of thirty-four landraces were studied for variation of morphological and agronomic traits following the IPGRI descriptors (IITA, BAMNET (2000)). Samples were also analysed with microsatellites markers which were characterised in this project. In addition DNA extracted using GenElute Plant Genomic DNA kit (Sigma Aldrich) were sent for DArT analysis. In addition, a total of 24 landraces that were previously selected from 17 clusters identified from the analysis of 223 bambaras

Materials and methods

line selection of bambara groundnut. diversity of 24 bambara groundnut landraces and to investigate employing SSR markers for pure study is to compare the use of morphological markers, DArT and SSR in assessing the genetic programmes and, potentially, direct selection via Marker Assisted Selection (MAS). The aim of this markers can then aid with the selection of germplasm for breeding, quality control within breeding decisions as to which marker is best to use in germplasm characterisation and plant breeding. Such The importance of a comparison of different marker systems is to assist in making informed line genetic variation.

markers showed S19-3 to be a genetically narrower (Mayes *et al.*, 2009), but still to have line-to-only one single genotype. An initial study on the comparison of S19-3 and Uniswared using SSR samples among 10 bambara groundnut landraces. Their results showed none of the landraces had one heterologous SSR primer pair, to investigate the intra-landrace variety using 10 to 15 individual plant of 250 pods. Singrun and Schenkel (2003) employed 10 primer combinations of AFLP and six lines of bambara groundnut landraces and reported a relatively high average number of pods per difficult in this species (Suwapraser *et al.*, 2006; Oyiga *et al.*, 2010). Wiggelsworth (1996) selected and effective way of bambara groundnut improvement especially given artificial hybridization is initial study was based on a limited number of SSR markers. Pure line selection could be a rapid descent could yield pure lines – essentially unselected varieties (Basu *et al.*, 2007), although this heterozygosity among bambara groundnut landraces suggests that selection based on single seed formal assessment of heterozygosity within bambara groundnut genotypes. Low levels of non-identical inbred lines, although the previous lack of co-dominant markers has prevented a Bambara groundnut is a predominantly self-pollinating crop so we would expect that it exists as sequence information to generate.

polymorphic and widely distributed in the genome (Tang *et al.*, 2006) but require substantial DArT are dominant markers thus has the disadvantage is to differentiate heterozygous loci from number of crops.

The comparison of markers in terms of their efficiency and reliability has been investigated in a cophenetic correlation matrix of (0.76), followed by morphological markers (0.38) and SSR (0.23). *ungiculata* spp. *sequepedalis*), their results showed ISSR markers to be more efficient with and morphological markers in assessing the genetic diversity of yard 28 yard long bean (*Vigna* SSR markers. Tantasawat *et al.*, (2010), compared the efficiency of SSR markers, ISSR markers resolution in cluster analysis compared to SSR markers in their analysis of 65 genotypes using 85 In common bean (*Phaseolus vulgaris*) Perugini *et al.*, (2010) found AFLP to provide better landraces in which a comparison with morphological markers is made has not yet been performed.

Basu et al., 2007) and of late DArT markers and morphological markers have been used (Oluolu BAPDs (Amadou *et al.*, 2001), AFLP (Massawe *et al.*, 2002; Ntundu *et al.*, 2003); SSR marker markers have been employed in bambara groundnut for genetic diversity assessment, such as genetic diversity in crops and estimate the level of heterozygosity in crops. A number of molecular assist breeders in developing new varieties since, they can be employed to thoroughly assess the (2008) described the morphological variation in bambara groundnut landraces. Molecular markers

groundnut landraces with 10 AFLP primer pairs of enzyme *EcoRI/MseI* and one heterologous SSR primer pairs (Singrun and Schenkel, 2003) were analysed.

DART markers: The procedures for generating DART markers, screening for polymorphism and genotyping was done by Diversity Arrays Pty Ltd., Yarralumla, Australia, following the methods described in Jaccoud *et al.*, (2001).

For pure line selections, seed derived from single plants were selected from the thirty four lines and planted in the field at Botswana College of Agriculture (Botswana) in the 2008/2009 season. To investigate the intra-landrace diversity, 7 individuals of the best five lines 81-Acc-385TZA, 84-Acc-696ZMB, 88-AHM-753NAM, S19-3NAM and Bots1-BWA, which produced higher pods numbers per plant and shoot dry weight were selected for molecular analysis. Seeds from the same individual lines were planted in a growth room in a drought response experiment in 2010 season at the University of Nottingham, making this the third season of selection of these lines. The effects of single plant selection on the genetic diversity based during three cropping seasons and selection is inferred, based mean and range of genetic distance estimates using (Popgene version 1.31; Yeh and Boyle 1997).

Microsatellite development: A microsatellite-enriched genomic library was constructed on the method of Kloda *et al.*, (2004), with some modifications and polymerase chain (PCR) reaction protocols are given in Basu *et al.*, (2007). Rather than cloning, a mixture of enriched libraries was pyrosequenced (Roche 454). Microsatellite primers were designed using PRIMER 3 (Rozen and Skaletsky, 2000) and were labelled using a three-primer tagged reaction (Schuelke, 2000). Seventy primer pairs that were designed and optimised for annealing temperature for 45°C to 60°C via an annealing temperature gradient (Hybaid PCR Express). A total of 65 microsatellites that produced clear amplification products were selected for further use. Individual samples of 24 bambara groundnut landraces representing genetic variability within the known germplasm were also characterised with the 65 microsatellites.

Morphological traits: 25 quantitative characters, days to emergence, days to flowering, leaf number per plant, plant canopy size, middle leaflet length, leaflet width, leaf area, plant height, internode length, petiole length, petiole-internode ratio, petiole width, peduncle length, number of stems, days to maturity, shoot dry weight, number of pods per plant, pod dry weight, pod width, pod length, number of seed per plant, seed length, seed width, shelling percentage and seed weight per plant were recorded in plants grown in 2008 in agromomy bay in UK and 2009 in a field experiment in Botswana. Data was collected based on bambara groundnut descriptor (IPGRL, 2000). 11 qualitative characters were recorded (leaf colour at emergence, pod texture, testa colour, eye pattern, stem hairiness, testa pattern, pod colour, pod shape, plant growth habit, leaflet colour, stem petiole colour). To reduce the effects of scale differences, quantitative characters were standardized using Genstat version 13.0 (Uphadyaya, 2003). The standardized values were used to perform cluster analysis and principal component analysis. The qualitative characters were recorded as binary data, for the absence of a trait as (0) and the presence of a trait as (1).

Data analysis: Genetic distances, cluster analysis, principal component analysis and Mantel tests were performed on the data. Pair-wise comparisons were estimated on Jaccard's similarity coefficient for each marker type. Morphological, DART, SSR dendrograms were constructed using UPGMA method of cluster analysis. The goodness of fit (matrix correlation) for genotypes to each cluster was estimated using cophenetic tests in NTSYS-pc software, version 2.1 (Rohlf, 1998). Cophenetic correlation is a procedure for evaluating hierarchical cluster techniques by comparing the input data for either similarity or dissimilarity with the output hierarchy (Hogersson, 1975). The similarities between markers were calculated based on the correlation between the similarity matrices for each marker type (Morphological marker vs SSR), (Morphological vs DART) and (SSR vs DART) and were tested on using a Mantel's test in the NTSYS software. The genetic distance between genotypes was estimated based on POPGENE version 1.31 (Yeh and Boyle, 1997), while the principal components analysis was calculated using MVSP (Kovach, 2006).

RESULTS AND DISCUSSIONS

The 65 SSR markers had an average polymorphic information content (PIC) of 0.46 among the 24 bambara groundnut landraces which was relatively higher, compared to the 201 DArT markers which detected an average PIC of 0.35 from the same genotypes (data not shown). The genetic diversity shown by the three markers in the selected 24 markers is relatively high (Table 1).

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Table 1: The estimates of genetic distance, pairwise range and principle component analysis among the 24 bambara groundnut landraces.

Markers type	Number of Markers used	Genetic distance		
		Average	Range ^a	Range ^b
Morphological	36	N/A	0.63-1.56	N/A
DArT	201	0.43	0.26-1.35	0.001-0.780
SSR	65	0.80	0.74-1.26	0.000-1.405
				25.43

a Jaccard genetic distance; b Nei's genetic distance on GenePop; c PCA % accumulation for first two Axes. N/A= not estimated

The polymorphism detected by both markers is in the range estimated in the earlier studies. Basu *et al.*, (2007) using 10 SSR markers detected an average PIC = 0.46 when analysing 18 bambara groundnut genotypes. In common bean (*Phaseolus vulgaris*), Buso *et al.*, (2006) observed a PIC = 0.56 among a set of 85 selected genotypes. Genetic diversity for the cultivated and wild relatives of pigeon pea using DArT marker recorded an average PIC of 0.34 (Yang *et al.*, 2006). SSR markers showed a higher genetic variability with a genetic distance (GD) ranging from 0 to 1.4 and an average of 0.80 as compared to DArT markers with a range of 0.001 to 0.780 and an average of 0.43. However, both the DArT markers and the morphological markers showed a better clustering as revealed by the Pairwise comparisons based on Jaccard similarity coefficients estimates. The DArT markers was more efficient in detecting the differences among the 24 landraces with a wide range difference of 1.09, followed by morphological marker with 0.9, and displaying least differences is the SSR marker at 0.52. The differences in genetic distance estimates by markers had been attributed to the extent of distribution of genome coverage by markers and their evolutionary different properties and individual loci used for analysis (Geleta *et al.*, 2005). The principal component analysis, using a combination of the first two axes shows that morphological markers (37.8%) were providing a better dispersion of the 24 bambara groundnut landraces, together with the DArT markers (35.8%), with SSR (25.43%) accounting for less genetic variation. Even though morphological data shows more variation, molecular data could also be used to predict the phenotypic diversity in crops and avoid a lot of field work (Motier *et al.*, 2005). Morphological data will also have a higher environmental dependency than DNA-based markers, which should have negligible genotypic, developmental or tissue effects.

Table 2: Cophenetic matrix correlations between morphological markers, DArT and SSR based on an analysis of the 24 bambara groundnut landraces

Morphology	DArT	SSR
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Correlations (r) of the similarity matrices are recorded above the diagonals, and the correlations (r) of the cophenetic matrices are recorded below the diagonal. The data in bold are the goodness of fit for each marker type.

	SSR		
Morphology	0.8224**	-0.004	0.0238
DART	-0.0187	0.9697**	0.2780
SSR	0.0518	0.1845	0.8386**

The co-phenetic matrix (goodness of fit) for all the markers was significant at $P < 0.01$, with morphological marker (0.82), DART (0.97) and SSR (0.84) (Table 2). Raman *et al.*, (2008) recorded a similar cophenetic coefficient of 0.97 for DART markers, in a set of 94 genotypes of *Lupinus albus* L. The Mantel tests for the comparison of the correlations of similarities matrices between the three markers were low, DART and morphological marker ($r = -0.004$), morphology and SSR ($r = 0.0238$), SSR and DART (0.2780) and were not significant. All the markers were below the suggested 0.5, which is considered statistically significant at ($P < 0.01$) (Lapointe and Lagendre, 1992). Low values were also reported in yard long bean (*Vigna unguiculata* ssp. *sequipedalis*) using 30 accessions, comparing SSR and morphological markers ($r = 0.1470$). Different results were observed by Stodart *et al.* (2006), between DART and SSR markers, since they recorded a strong positive correlation ($r = 0.84$), when using 256 DART markers and 63 SSR markers on 44 accessions of bread wheat (*Triticum aestivum* L.). Mantovani *et al.* (2008) found a coefficient correlation between the genetic distance matrices of DART and SSR ($r = 0.68$) among a set of 31 accessions of wheat using 1,315 DART markers and 103 SSR markers, which also showed an agreement between the two markers.

The dendrograms from the UPGMA cluster analysis for the morphological, DART and SSR markers are recorded in figure 1, figure 2 and figure 3, respectively. The morphological markers revealed two major clusters with landraces separated mainly on the characters which contribute more variation in barnbara groundnut such as leaf area, shoot dry weight, and number of pods per plant. Landraces clustered together are more likely to have a similar performance in a specific environment. Cluster 2 consists of mostly landraces from Southern Africa with the exception of landraces, Tvsu 610 from Nigeria, DodR from Tanzania and Ramayana from Indonesia, which also performs equally well in Southern Africa or perhaps arguing that they have been introduced by farmers from other regions

DART and SSR markers are showing a similar pattern as each produced 3 clusters. The 201 DART markers separated samples particularly based on their areas of origin (Figure 2). DodC and DodR from Tanzania in the cluster 1 grouped together, while cluster 2 consists of landraces originally from Southern Africa, with the exception of Ramayana 'currently' from Indonesia. This could also reflect the genetic relation the Indonesian landraces from Southern Africa and a potential place of origin from Africa. The third cluster consists of landraces only from East Africa. As for the SSR marker, the dendrogram was produced from 65 SSR markers and identified three major groups. The clustering is not based on the areas of origin of landrace, since both clusters are showing some mixtures of landraces from other regions. However, like the morphological markers the clusters consist of many landraces which do come from the same region.

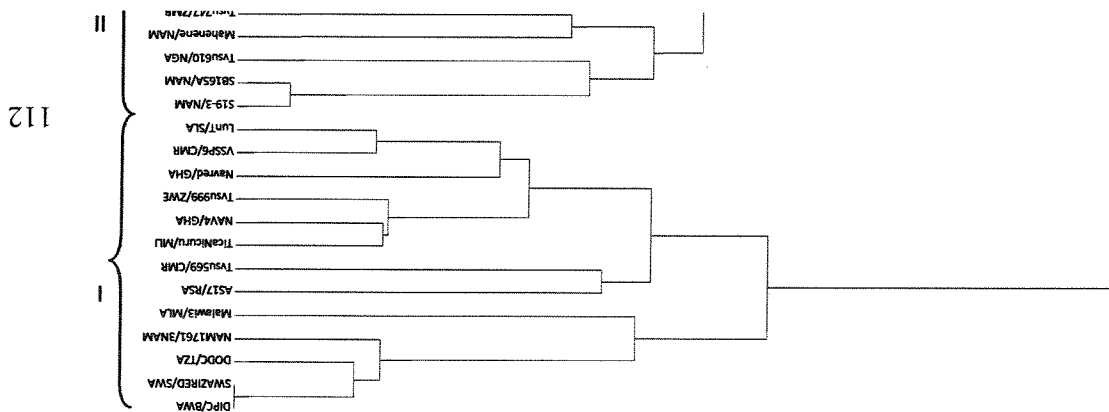
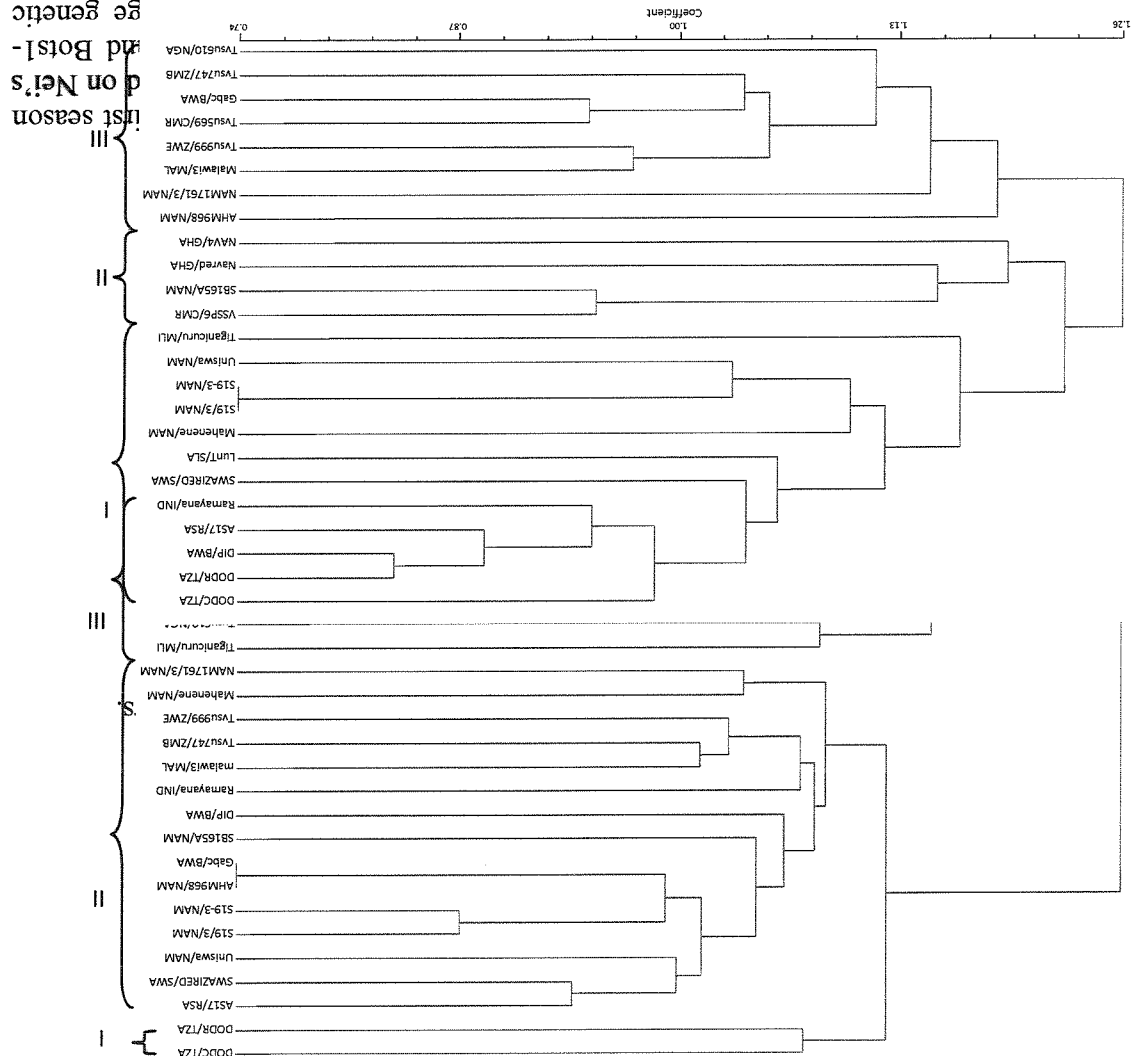


Figure 1: UPGMA dendrogram for the 24 bambara groundnut landraces based on 26 morphological markers.



distance for lines 84-AHM753NAM (0.00), 88-Acc696ZMB (0.006) and S19-3NAM (0.016) revealed that they were genetically narrower compared to Botsi (0.10) and 81-Acc696ZMB (0.28) in the first cycle of selection (Table 3). Variability within bambara groundnut has been reported before in landraces, and has been attributed to the mixing of seeds during planting, especially those of same colour (Massawe *et al.*, 2003; Mayes *et al.*, 2009) although even within landraces, there are still differences between lines.

As a breeding strategy for inbreeding crops like bambara groundnut, it is advantageous to obtain pure homozygous lines with good attributes. As expected, in the second and third round of selection for pure lines were selected through single plant (Table 3). There was no observed or expected heterozygosity in the second and third round of selection. This data strongly suggest these genotypes are now relatively pure lines.

Table 3: Mean and range of the genetic distances values for three different selection cycles of bambara groundnut from single seed descent estimated based on 12 microsatellites markers using POPGENE version 1.31 (Yeh and Boyle, 1997).

Genetic distance estimates											
First cycle selection				Second cycle selection				Third cycle selection			
Selected lines	N	Mean	Ho-He	Range	N	Mean	Ho-He	Range	N	Mean	Ho-He
81-Acc3857ZA	3	0.284	(0.00)0.33	0.187-0.417	7	0.00	0.00	0.00	6	0.00	0.00
84-Acc 696ZMB	3	0.000	(0.00)0.00	0.00-0.00	4	0.00	0.00	0.00	6	0.00	0.00
88-AHM 753NAM	3	0.006	(0.00)0.08	0.05-0.102	7	0.00	0.00	0.00	6	0.00	0.00
S19-3NAM	3	0.016	(0.00)0.00	0.00-0.024	7	0.00	0.00	0.00	6	0.00	0.00
Boei BWA	3	0.103	(0.00)0.17	0.05-0.158	7	0.00	0.00	0.00	6	0.00	0.00
N = Number of individual samples											

CONCLUSIONS

The study has demonstrated the utility of the three markers in genetic diversity and relatedness among the 24 bambara groundnut landraces, since both markers recorded a significantly higher cophenetic correlation matrix ($P < 0.01$). The markers provided consistent information as they produced relatively similar dendrogram, but DART markers producing a more robust cluster which grouped landraces based on their areas of origin. However, it was the SSR markers which revealed a higher genetic diversity among the landraces with a larger average genetic distance and range based on Nei's genetic distance. The poor correlation of the similarity matrix for the three markers showed that, these markers target different part of the genome. SSR markers were used in the investigation of pure line selection of bambara groundnut, the results strongly suggests that there is level of heterozygosity is significantly reduced in the second round of selection, with no signs of residual heterozygosity in the third cycle of selection. The application of SSR markers in this study demonstrates their effectiveness as a technique suitable technique for Marker Assisted Selection in bambara groundnut.

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