Genetic Improvement of Grain Yield and Quality in Rice *(Oryza sativa* L.*)* in South Sudan

by

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Thesis Abstract

The development of new high yielding varieties may not generate considerable impact unless the selection takes into account end-user qualities. Whereas consumer choices of rice varieties are largely based on grain cooking and eating qualities, improving grain quality and yield are important considerations but present a major challenge for most rice breeding programmes in sub-Saharan Africa. Therefore, understanding the factors that contribute to enhanced yield and grain quality as a basis for breeding and selection strategies in rice is important. Thus, the objectives of this study were: 1) to investigate farmers' perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated rice agro-ecologies in South Sudan, to guide breeding and policy interventions; 2) to perform genetic and comparative analysis of agro-morphological and grain quality traits of elite upland rice genotypes; 3) to investigate genetic diversity and perform genome-wide association studies of grain quality traits in a panel of 59 upland and lowland rice; 4) to determine genetic variability and to identify QTLs for yield-related and grain quality traits in three rice F_2 populations. These studies were conducted at various sites in South Sudan, Uganda and Kenya.

The first study investigated farmers' perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated rice agro-ecologies in South Sudan. Data were gathered through participatory rural approaches and a formal structured survey involving 136 rice farmers from major rice growing areas of South Sudan. Farmers predominantly cultivated old rice varieties with low yields varying from 0.4 to 1.6 t ha^{-1}. Yield, early maturity, cooking and eating quality, nutritional importance and drought tolerance were the most desirable traits of rice ideotypes. Further, imported rice varieties were best ranked for their sweet and appealing taste, rich aroma, grain shape and size, swelling capacity and non-stickiness during cooking. Major perceived constraints to rice production were unreliable rainfall, poor access to credit facilities, poor soil and water management practices, poor rice storage facilities, inadequate and poor processing machines as well as limited technical skills in rice production.

The second study assessed the genetic diversity and relationship present among 36 elite upland rice genotypes using Diversity Arrays Technology Sequencing (DArTseq) markers and agro-morphological and grain quality traits. Genetic diversity estimates amongst test genotypes revealed only two distinct clusters suggesting genetic relatedness. This was associated with a low mean fixation index (F_{st}) of 0.188. Analysis of variance based on agro-morphological and grain quality traits indicated highly significant differences ($P<0.001$) among the tested genotypes. Principal component and cluster analyses indicated that major contributors to grain yield per plant were, number of productive tillers per plant, number of panicles per plant, grain width, number of grains per panicle, panicle length, grain length to width ratio, percent spikelet fertility and weight of 1000 grains. Grain yield had significant positive correlation with number of panicles per plant, percent spikelet fertility and number of grains per panicle. Path coefficient analysis showed that direct selection for number of grains per panicle can be effective in enhancing grain yield. Grain length expressed the greatest maximum direct positive effect on amylose content, while grain width revealed the largest negative effect on this trait. Number of grains per panicle, number of panicles per plant, weight of 1000 grains, percent spikelet fertility and grain length can be considered as important selection criteria for genetic improvement of grain yield and cooking quality in rice.

The third study performed genetic diversity and genome-wide association studies of grain quality traits in a collection of 59 upland and lowland rice using DArTseq markers. Population structure analysis revealed only two distinct genetic clusters which grouped genotypes based on environmental adaptation and pedigree information. Analysis of molecular variance indicated a low degree of differentiation among populations suggesting the need for broadening the genetic base of the current germplasm collection. GWAS revealed 22 significant associations between DArTseq-derived SNP markers and rice grain quality traits in the test genotypes, two of which were in chromosomal regions where the QTLs associated with the given traits had previously been reported, while the other 20 were indicative of novel alleles for rice grain quality traits. DArTseq-derived SNP markers that included SNP12_100006178, SNP13_3052560 and SNP14_3057360 individually co-localised with two functional gene groups that were associated with QTLs for grain width and grain length to width ratio on chromosome 3, indicating trait dependency or pleiotropic-effect loci.

The fourth study determined genetic variability and identified QTLs for yield-related and grain quality traits in three rice F_2 populations developed from a cross between *O.sativa* L. rice variety (improved grain quality traits but low yield) and an interspecific hybrid [(*O. sativa* L. x *O. glaberrima* Steud), improved yield but poor grain quality traits] using DArTseq-derived SNP markers. From a high-density genetic linkage map constructed, a total of 23 main-effect quantitative trait loci (M-QTLs) were detected for grain length, grain width, amylose content and alkali spreading value. High levels of transgressive segregation for yield-related and grain quality traits observed indicated the potential for improvement of these economically important traits. Moreover, the detection of novel M-QTLs that enhanced grain quality underscores the potential value of the germplasm used in the present study as a useful source for grain yield and quality improvement. M-QTLs that mapped to regions consistent with map locations in other studies can be useful in marker-assisted selection experiments.

The results showed a need for integration of farmers' and stakeholders' preferences in variety development process and the desirability of releasing site-specific rice cultivars across major rice growing areas of South Sudan. Overall, using DArTseq-derived SNP markers and agro-morphological and grain quality traits resulted in selection of the following genetically diverse upland rice genotypes: 'P5H6', 'NAMCHE 6' and 'ART3-7L9P8-3-5-B-B-2' for direct production or breeding. Upland and lowland rice genotypes including; 'K5', 'ART2-4L3P1-2-1', 'BG400-1', 'JARIBU', 'SUPA TZ', 'BR4' and 'ART3-8L6P3-2-2-B', were identified as potential sources of major effect QTLs for grain quality traits that can be exploited for rice crop improvement. Populations 'Supa 1052 x NERICA 4' and 'Komboka x NERICA 4' recorded the highest mean grain yield and intermediate AC and ASV. The aforementioned crosses also recorded the highest number (22) of main-effect QTLs (M-QTLs) detected among their F_2 mapping populations out of a total of 23 M-QTLs that were uncovered for grain length, grain width, amylose content and alkali spreading value. These crosses can be useful for selection of desirable segregates at the F_2 and BC_1F_2 generations. M-QTLs that mapped to regions consistent with other studies may be useful in marker-assisted selection experiments. Novel M-QTLs detected in this study for yield-related and grain quality traits could serve as candidates for future fine-mapping and positional cloning projects.

Declaration

I, Lado Maurice Mogga, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

   i) Their words have been re-written but the general information attributed to them has been referenced.

   ii) Where their exact words have been used, their writing has been placed in italics and inside quotation marks and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference sections.

Signed:

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As the candidate's supervisors we agree to the submission of this thesis

Sibiya

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Dr. Julia Sibiya (Supervisor)

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Professor Hussein Shimelis (Co-Supervisor)

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Dedication

This thesis is dedicated to the people who have always believed in me; to my father (Ben Lado) and mother (Mary Lado) who are the primary cornerstones of my education journey, my wife (Senit) and sons (Ian) and (Aaron), being away from them during the course of the study required a lot of patience, understanding and sacrifices.

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Introduction to Thesis

1 Background

Rice (*Oryza sativa* L.) is the second most important and widely grown cereal crop after wheat, providing over 21% of the calorific needs of the world's population (Lu et al., 2013). It occupies 11% of the world's total arable land (Tehrim et al., 2012) and its increased production in the past four decades has been attributed to the adoption of the Green Revolution technologies for rice intensification (Mba et al., 2012). Furthermore, with the current growth in population, increased urbanization and consumer preferences, particularly in Africa and Latin America; there has been a growing global demand for high quality rice (Balasubramanian et al., 2007). This demand offers a better opportunity for rice growing countries to reduce rice imports and generate higher export revenues. In addition, increased urbanization and changes in consumer preferences also explains the gradual shift in rice consumption patterns from traditional coarse grains like maize, millet and sorghum, to non-traditional grains like wheat and rice (Demont, 2013). Rutsaert et al. (2011) indicated that the demand for rice in Africa has increased significantly at an average annual rate of 4.4%, to attain a total consumption level of 20 million tonnes in 2009. However, Africa's rice sector has not been able to match the increased growth in demand for high quality rice and as a result it has become increasingly dependent on rice imports (Seck et al., 2010; Demont, 2013).

In sub-Saharan Africa (SSA), close to 40% of the rice consumed is imported (Seck et al., 2010) and the trend has been growing at a much faster rate than production (Figure 1.1). In 2007, the total quantity of milled rice consumed in Africa was estimated at 25 million tonnes with an average per capita consumption of 24 kg per year. In the same year, paddy rice production in Africa was 23.4 million tonnes and 9.7 million tonnes were imported (Seck et al., 2010). Given the foregoing, an increased dependence on rice imports exposes countries in SSA to international market shocks and uncertainties. Hence, this may have a direct impact on food security and political stability (Becker, 2009; Moseley et al., 2010; Seck et al., 2010; Dupraz and Postolle, 2013).

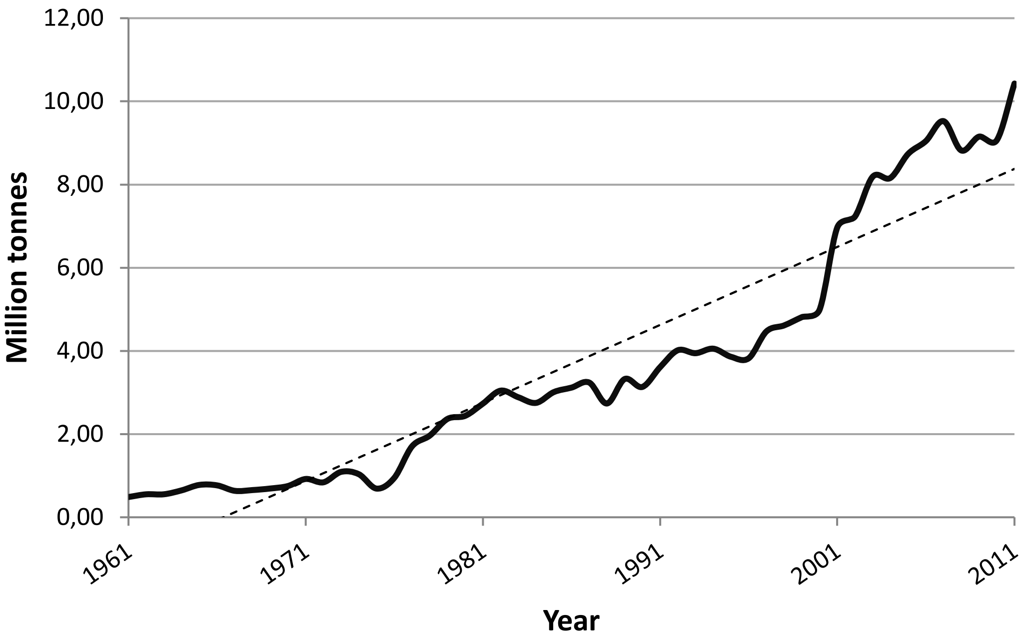


Figure 1.1: Rice imports (million tonnes) in sub-Saharan Africa from 1961–2011 (FAO, 2014).

2 Importance of rice in South Sudan and farmer preferences

Rice in South Sudan is ranked among the first four dominant staple cereals after sorghum, maize, and millet in terms of consumption. Results from the 2009 National Baseline Household Survey (NBS, 2012) suggested that more than 75% of rural households consume cereals. Furthermore, in the last two decades, South Sudan (formerly Sudan), was a net exporter of agricultural produce to regional markets and the potential of agriculture in stimulating economic growth was emphasized. However, due to civil war-related destruction, poor infrastructure and lack of investment in the agriculture sector, South Sudan is now a net importer of food. It currently imports as much as 50% of its food needs, including 40% of its cereals from neighbouring countries, particularly Uganda and Kenya. Total food imports are estimated to be in the range of US$ 200-300 million a year (AfDB, 2013).

It is also estimated that South Sudan receives all its rice imports through Uganda (Nzomoi and Anderson, 2013), which acts as an important transit corridor for rice shipment of both locally produced and imported rice. Despite the importance of rice in South Sudan, production is low, while introduced varieties do not meet the standards of imported rice in physical, cooking and eating qualities. Therefore, these varieties do not offer competitive prices in the market. A major challenge in the rice sector in South Sudan is, therefore, how to produce sufficient and affordable high quality rice that not only meets the preferences of its fast-growing and increasingly urbanized population, but also competes favourably with imported rice.

In addition, consumer preferences may be variable and depend partly on historical and socio-cultural factors, such that quality rice in one region may be considered low-quality in another. A study by Nzomoi and Anderson (2013) on rice markets in East Africa suggested that released rice varieties are not widely adopted because, in most cases, farmers and consumers may not prefer the taste or aroma of the introduced variety. For example in Uganda, consumers prefer aromatic to non-aromatic rice, sticky to non-sticky rice, unbroken to broken, bulging after cooking to rice that does not bulge, and white milled rice to brown (MAAIF, 2012). Additionally, during a pre-survey study on rice in South Sudan (MAFCRD, 2013), farmers identified high yielding, improved grain quality and early maturity as critical factors in selection of new rice varieties. Grain quality traits are, therefore, important considerations for all individuals involved in production, processing and consumption of rice, and hence dictate the nutritional and commercial value of rice grain (Koutroubas et al., 2004; Fitzgerald et al., 2009).

Grain quality is usually based on certain objective or subjective criteria that are of relative importance to the end-user. In general, the most important grain quality traits that are common to all end-users include appearance, milling quality, cooking, processing quality and nutritional quality (Lou et al., 2009). In light of this, knowledge on end-user tastes and preferences for rice grain quality traits is important for enhancing rice breeding strategies. This will ensure competitiveness of locally produced rice and thus serve as a mechanism to return more of the benefits associated with improved quality rice to the farmer.

3 Rice breeding in sub-Saharan Africa

For most African countries, the focus in rice breeding programmes has been on increasing yield and enhancing stress tolerance with little emphasis on grain eating and cooking qualities (Manful, 2010). High yielding varieties are often released after field-testing without taking into account their quality attributes such as milling potential, amylose content, gelatinization temperature, gel consistency, chalkiness, pasting characteristics, cooked rice texture and aroma. Furthermore, consumer tastes and preferences are rarely incorporated in the selection of new rice varieties. These varieties may satisfy the farmers' preferences but not the end-user qualities, thus the locally produced rice is unable to compete with imported rice that has improved cooking and eating qualities. Therefore, there is an increasing importance for enhanced yield and end-user quality in the local rice varieties. In addition, it is essential to broaden the genetic base of rice genotypes by introducing genes from distant or wild relatives with potential for delivering novel genes or quantitative trait loci (QTLs) for important agronomic traits. The magnitude of genetic variability and the extent to which the desirable characters are heritable largely determines the success of any plant breeding programme (Vanaja and Luckins, 2006).

However, breeding for improved cooking and eating quality traits in rice has several challenges such as polygenic inheritance and genotype-by-environment interactions (Lapitan et al., 2007; Ordonez et al., 2010). In addition, the complexity in accurate evaluation of cooking and eating quality at early breeding generations has constrained the development of rice varieties with enhanced cooking and eating qualities (Lestari et al., 2009). Furthermore, yield and nutritional quality are said to be negatively correlated (Hussain et al., 2010). However, previous reports (Swamy and Sarla, 2008; Swamy and Sarla, 2011; Swamy et al., 2012) indicated several eating and cooking quality quantitative trait loci (QTLs) that were associated with major QTLs for yield-related traits in rice. They suggested that the major effective yield-enhancing QTLs from *O. nivara* (a wild progenitor of Asian cultivated rice *O. sativa*) could possibly be introgressed individually or pooled selectively without compromising grain quality in Swarna (a rainfed lowland rice cultivar) (Swamy et al., 2012).

More recently with the advances in next generation sequencing (NGS) technologies, genotyping by sequencing (GBS) has emerged as a promising genomic approach for simultaneous exploration of plant genetic diversity and molecular marker discovery (Poland and Rife, 2012; He et al., 2014). Thus, GBS has effectively been used for single-nucleotide polymorphisms (SNP) marker discovery and QTL identification of tightly linked marker-trait associations (Tang et al., 2016) and in the application of genomic selection of complex traits for crop improvement (Jarquín et al., 2014). The GBS approach is, therefore, considered an important cost-effective tool for population genetics, QTL discovery, high-resolution mapping and for genomic selection in plant breeding programmes (Furuta et al., 2017). Thus, given these developments, several markers and QTLs affecting rice grain yield and quality have been identified and mapped in various mapping populations (McCouch et al., 2002; Xing and Zhang, 2010), in order to apply marker-assisted selection for enhancing breeding efficiency. However, the identified QTLs may not be sufficient to elucidate the genetic basis of grain yield and quality. Also, the varied nature of grain yield and quality traits (Liu et al., 2010) underscore the need for identifying novel QTLs in order to design a breeding strategy for their improvement. Consequently, with the accumulated information on QTLs for grain yield and quality traits and their closely linked markers (Sattari et al., 2015), QTL pyramiding can be applied as a direct approach for improving the target traits.

Accordingly, other successful studies in breeding for enhanced yield and grain quality have been reported, most notably for maize (*Zea mays* L.) in developing ''Quality Protein Maize'' (QPM) which is opaque-2 maize that has been selected for enhanced grain characteristics and enhanced yield using marker assisted breeding (MAB) (Vasal, 2002). Marker-assisted selection (MAS) has also been used to enhance grain quality traits of a male sterile line and maintainer line widely used in hybrid rice breeding in China (Zhou et al., 2003; Jin et al., 2010) and to improve cooking quality traits in Myanmar rice cultivar Manawthukha (Yi et al., 2009). Thus, with the use of DNA-based molecular markers such as Single-nucleotide Polymorphisms (SNPs) in MAS, it is possible to overcome the challenges of dealing with complex quantitative traits and concomitant introgression of more than one targeted QTL into an elite crop variety (Collard and Mackill, 2008; Mir and Varshney, 2012; Gupta et al., 2013; Tyagi et al., 2014). Therefore, a better understanding of the factors that contribute to improved grain yield and quality of rice will lay the foundation for developing new breeding and selection strategies for generating rice cultivars that combine yield and end-user qualities. Also, MAS can significantly improve the efficiency and effectiveness of conventional breeding (Gupta et al., 2010). This is necessary for South Sudan to meet the growing domestic demand for high quality rice while offering the country with additional opportunities for generating higher export revenues.

4 Justification of the current study

South Sudan has a great potential for agriculture with more than 50% of its arable land mass as prime agricultural land and yet less than 5% is currently utilised (AfDB, 2013). Two out of the six agro-ecological zones (the Flood plains and Greenbelt agro-ecological zones) of South Sudan are most suitable for rice cultivation and constitute 70% of the arable land. Rice is cultivated under two production systems that include the rainfed upland (in the Greenbelt agro-ecological zone mainly in Yambio, Yei and Morobo districts/counties) and irrigated lowland (in the flood plains agro-ecological zone mainly in Aweil North county/Aweil rice scheme). The latter provides the greatest potential (80%) for lowland rice cultivation. Despite the huge potential for rice expansion and the constant rise in demand, less than 5000 hectares of land is utilized for rice cultivation, which is by far insufficient to meet the local demand, thus encouraging increased rice imports. Additionally, the long period of civil war in South Sudan derailed the establishment of rice research, while efforts by non-governmental organizations (NGOs) were largely centred on introductions of improved varieties for use by farmers. Unfortunately, these varieties do not go through testing for quality, adaptation and other agronomic traits. Thus, farmers take risk in planting varieties of unknown performance that are largely low yielding and susceptible to diseases and other biotic/abiotic stresses. Furthermore, these varieties may not meet farmers' or end-user qualities and preferences, and therefore cannot compete favourably with imported rice. For example, farmers may prefer high yielding and disease resistant varieties, while consumers may select varieties with improved cooking and eating qualities. A combination of both farmer and end-user qualities in one genetic background is desirable but remains a major challenge in a rice breeding programme. Therefore, breeding for enhanced yield and end-user quality would be an important key strategy for South Sudan's rice breeding programme to further accelerate rice growth and development in the country. Consequently, increased rice production and market competiveness of locally produced rice would contribute to food, income and nutrition security for South Sudan. Thus, the overall objective of the study is to provide an understanding of the factors that contribute to enhanced yield and grain quality traits as a basis for breeding and selection strategies in rice germplasm for South Sudan.

5 Research objectives

The specific objectives of the study were;

i. To investigate farmers’ perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated rice agro-ecologies in South Sudan to guide breeding and policy interventions
ii. To perform genetic and comparative analysis of agro-morphological and grain quality traits of elite upland rice genotypes.
iii. To investigate genetic diversity and perform genome-wide association studies of grain quality traits in a panel of 59 upland and lowland rice collections.
iv. To determine genetic variability and to identify QTLs for yield-related and grain quality traits in three rice F_2 populations involving crosses of *O. sativa* L. and interspecific (O*. sativa* L. x *O. glaberrima,* Steud) hybrid.

6 Research hypotheses

The following hypotheses were tested;

i. Farmers and stakeholders are aware of major rice production constraints and their needs and preferences in a rice variety in South Sudan.
ii. There exists wide variability in agro-morphological and grain quality traits among upland rice germplasm collection in South Sudan with potential use for production and crop improvement.
iii. Rice populations in South Sudan are diverse and ideal for identifying significant marker-trait associations between grain quality traits and DArTseq-derived SNP markers.
iv. Adequate genetic variability exists within mapping populations involving *O.sativa* and interspecific hybrid (*O.sativa* L. x *O.glaberrima,* Steud), while their hybridization can be successfully exploited to improve grain yield and quality in rice.

7 Outline of the thesis

This thesis is made up of five individual chapters that reflect the set objectives for this research study. Chapter 1 is written as a separate review paper, while chapters 2 to 5 are written as discrete research papers, each following the format of a stand-alone research paper in accordance with the standard thesis format adopted by the University of KwaZulu-Natal. Thus, there is some unavoidable repetition of references and introductory remarks between chapters. Chapter 6 gives a general overview and implications of findings from the study. The Crop Science referencing system was used in all chapters of this thesis. Therefore, the thesis comprised the following chapters:

1. Thesis Introduction
2. Chapter 1: Literature review
3. Chapter 2: Appraisal of major determinants of rice production and productivity and farmers' choice of ideotypes in South Sudan: implications for breeding and policy interventions
4. Chapter 3: Genetic analysis of elite upland rice genotypes using DArTseq markers and comparative analysis of agro-morphological and grain quality traits
5. Chapter 4: Diversity analysis and genome-wide association studies of grain quality traits in rice (*Oryza sativa* L.) using DArTseq markers
6. Chapter 5: Genetic variability and quantitative trait loci analysis for yield-related and grain quality traits in three rice F_2 populations
7. Chapter 6: General overview and implications of the study

8 References

AfDB. 2013. African Development Bank Group.South Sudan: An Infrastructure Action Plan - A Program for Sustained Strong Economic Growth. p. 131-167.

Balasubramanian, V., M. Sie, R.J. Hijmans and K. Otsuka. 2007. Increasing Rice Production in Sub-Saharan Africa: Challenges and Opportunities. Advances in Agronomy. 94: 55-133.

Becker, L. 2009. Rice Producer-Processor Networks in Côte D'ivoire. Geographical Review 99: 164-185.

Collard, B.C. and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 557-572.

Demont, M. 2013. Reversing urban bias in African rice markets: A review of 19 National Rice Development Strategies. Global Food Security. 2(3): 172-181.

Dupraz, C.L. and A. Postolle. 2013. Food sovereignty and agricultural trade policy commitments: How much leeway do West African nations have? Food Policy 38: 115-125.

FAO. 2014. FAOSTAT agriculture data. Available at http://faostat3.fao.org. Accessed on April 12th, 2014.

Fitzgerald, M.A., S.R. McCouch and R.D. Hall. 2009. Not just a grain of rice: the quest for quality. Trends in Plant Science 14: 133-139.

Furuta, T., M. Ashikari, K.K. Jena, K. Doi and S. Reuscher. 2017. Adapting Genotyping-by-Sequencing for Rice F2 Populations. G3: Genes| Genomes| Genetics: g3. 116.038190.

Gupta, P., P. Langridge and R. Mir. 2010. Marker-assisted wheat breeding: present status and future possibilities. Molecular Breeding 26: 145-161.

Gupta, P.K., S. Rustgi and R.R. Mir. 2013. Array-based high-throughput DNA markers and genotyping platforms for cereal genetics and genomics. Cereal Genomics II. Springer. p. 11-55.

He, J., X. Zhao, A. Laroche, Z.-X. Lu, H. Liu and Z. Li. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in Plant Science. 5: 484.

Hussain, S., M.A. Maqsood and M. Rahmatullah. 2010. Increasing grain zinc and yield of wheat for the developing world: A Review. Emirates Journal of Food and Agriculture 22(5): 326.

Jarquín, D., K. Kocak, L. Posadas, K. Hyma, J. Jedlicka, G. Graef and A. Lorenz. 2014. Genotyping by sequencing for genomic prediction in a soybean breeding population. BMC Genomics. 15(1): 740.

Jin, L., Y. Lu, Y. Shao, G. Zhang, P. Xiao, S. Shen, H. Corke and J. Bao. 2010. Molecular marker assisted selection for improvement of the eating, cooking and sensory quality of rice (*Oryza sativa* L.). Journal of Cereal Science 51(1): 159-164.

Koutroubas, S.D., F. Mazzini, B. Pons and D.A. Ntanos. 2004. Grain quality variation and relationships with morpho-physiological traits in rice (*Oryza sativa* L.) genetic resources in Europe. Field Crops Research 86: 115-130.

Lapitan, V.C., D.S. Brar, T. Abe and E.D. Redoña. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. Breeding Science. 57(4): 263-270.

Lestari, P., T.H. Ham, H.H. Lee, M.O. Woo, W. Jiang, S.H. Chu, S.W. Kwon, K. Ma, et al. 2009. PCR marker-based evaluation of the eating quality of japonica rice (*Oryza sativa* L.). Journal of Agricultural and Food Chemistry. 57(7): 2754-2762.

Liu, X., X. Wan, X. Ma and J. Wan. 2010. Dissecting the genetic basis for the effect of rice chalkiness, amylose content, protein content, and rapid viscosity analyzer profile characteristics on the eating quality of cooked rice using the chromosome segment substitution line population across eight environments. Genome 54: 64-80.

Lou, J., L. Chen, G. Yue, Q. Lou, H. Mei, L. Xiong and L. Luo. 2009. QTL mapping of grain quality traits in rice. Journal of Cereal Science 50: 145-151.

Lu, L., S. Tian, H. Liao, J. Zhang, X. Yang, J.M. Labavitch and W. Chen. 2013. Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. PLoS ONE. 8(2): e57360.

MAAIF. 2012. Ministry of Agriculture Animal Industry and Fisheries, Uganda National Rice Development Strategy (NRDS), 2008-2018. p. 8-12.

MAFCRD. 2013. Ministry of Agriculture, Forestry, Cooperatives, Rural and Development. Participatory rice breeding for impact in South Sudan. Unpublished.

Manful, J. 2010. Developing rice varieties with high grain quality in Africa: Challenges and prospects. Second Africa Rice Congress, Bamako, Mali, 22–26 March 2010: Innovation and Partnerships to Realize Africa's Rice Potential.

Mba, C., E.P. Guimaraes and K. Ghosh. 2012. Re-orienting crop improvement for the changing climatic conditions of the 21st century. Agriculture and Food Security. 1(1): 7.

McCouch, S., L. Teytelman, Y. Xu, K. Lobos and K. Clare. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Research. 9: 199-207.

Mir, R.R. and R.K. Varshney. 2012. Future prospects of molecular markers in plants. Molecular Markers in Plants: 169-190.

Moseley, W.G., J. Carney and L. Becker. 2010. Neoliberal policy, rural livelihoods, and urban food security in West Africa: A comparative study of the Gambia, Côte d'Ivoire, and Mali. Proceedings of the National Academy of Sciences. 107(13): 5774-5779.

NBS. 2012. South Sudan National Bureau of Statistics. National Baseline Household Survey, 2009. Report for South Sudan. p. 175.

Nzomoi, J. and I. Anderson. 2013. The Rice Market in East Africa. Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists, September 22-25, 2013,Hammamet,Tunisia. p. 14.

Ordonez, S.A., J. Silva and J.H. Oard. 2010. Association mapping of grain quality and flowering time in elite japonica rice germplasm. Journal of Cereal Science. 51(3): 337-343.

Poland, J.A. and T.W. Rife. 2012. Genotyping-by-sequencing for plant breeding and genetics. The Plant Genome 5: 92-102.

Rutsaert, P., M. Demont and W. Verbeke. 2011. Consumer preferences for rice in Africa. Realizing Africa's rice promise. CAB International. p. 218-229.

Sattari, A., N. Mahdinezhad, B. Fakheri, M. Noroozi, and H. Beheshtizadeh. 2015. Improvement of the eating and cooking qualities of rice: a review. International Journal of Farming and Allied Sciences.*4* (2), pp.153-160.

Seck, P.A., E. Tollens, M.C.S. Wopereis, A. Diagne and I. Bamba. 2010. Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. Food Policy 35: 403-411.

Swamy, B., K. Kaladhar, N. Shobha Rani, G.S. Prasad, B. Viraktamath, G. Reddy and N. Sarla. 2012. QTL analysis for grain quality traits in 2 BC2F2 populations derived from crosses between *Oryza sativa* cv Swarna and 2 accessions of *O. nivara*. Journal of Heredity. 103(3): 442-452.

Swamy, B. and N. Sarla. 2008. Yield-enhancing quantitative trait loci (QTLs) from wild species. Biotechnology Advances 26: 106-120.

Swamy, B. and N. Sarla. 2011. Meta-analysis of yield QTLs derived from inter-specific crosses of rice reveals consensus regions and candidate genes. Plant Molecular Biology Reporter. 29(3): 663-680.

Tang, W., T. Wu, J. Ye, J. Sun, Y. Jiang, J. Yu, J. Tang, G. Chen, et al. 2016. SNP-based analysis of genetic diversity reveals important alleles associated with seed size in rice. BMC Plant Biology. 16(1): 93.

Tehrim, S., Z.H. Pervaiz, M.Y. Mirza, M.A. Rabbani and M.S. Masood. 2012. Assessment of phenotypic variability in rice (*Oryza sativa* L.) cultivars using multivariate analysis. Pakistan Journal of Botany. 44(3): 999-1006.

Tyagi, S., R. Mir, H. Kaur, P. Chhuneja, B. Ramesh, H. Balyan and P. Gupta. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). Molecular Breeding. 34(1): 167-175.

Vanaja, T. and T.B. Luckins. 2006. Variability in grain quality attributes of high yielding rice varieties (*Oryza sativa* L.) of diverse origin. Journal of Tropical Agriculture. 44(1-2): 61-63.

Vasal, S. 2002. Quality protein maize: overcoming the hurdles. Journal of Crop Production. 6(1-2): 193-227.

Xing, Y. and Q. Zhang. 2010. Genetic and molecular bases of rice yield. Annual Review of Plant Biology. 61: 421-442.

Yi, M., K.T. Nwe, A. Vanavichit, W. Chai-arree and T. Toojinda. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113: 178-186.

Zhou, P., Y. Tan, Y. He, C. Xu and Q. Zhang. 2003. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theoretical and Applied Genetics. 106(2): 326-331.

1. Literature review

Abstract

The development of new high yielding varieties may not generate considerable impact unless the selection takes into account end-user qualities. Although yield relates to the most important trait of farmer-preferred cultivar, this does not always translate into increased revenue since consumers' taste and preferences and market-demand are often considered a major driver of widespread uptake of a new variety. Given that consumers' choice of rice varieties are largely based on grain cooking and eating qualities, improving grain quality and yield are important considerations but present a major challenge for most rice breeding programmes in sub-Saharan Africa. This review examines the current challenges and prospects in rice breeding for improved grain yield and quality. Furthermore, it provides a critical analysis of significant developments in genetic and molecular understanding of grain yield and quality in rice while highlighting on the potential research gaps and way forward.

Keywords*:* Rice, Grain yield and quality, Farmer and consumer preferences, Conventional breeding approaches, Marker-assisted selection, Genomics-assisted breeding,

1.1 Introduction

Rice is the second most widely grown cereal crop and a primary staple food for more than half of the world's population (Siddiq et al., 2012). With the current growth in population particularly in Africa and Latin America, increased urbanization and changes in consumer preferences, there has been a growing demand for high quality rice (Balasubramanian et al., 2007). According to Rutsaert et al. (2011), the demand for rice in Africa has increased significantly at an average annual rate of 4.4% to attain a total consumption level of 20 million tons in 2009. Furthermore, in sub-Saharan Africa (SSA), close to 40% of the rice consumed is imported (Seck et al., 2010) and the trend has been growing at a much faster rate than production (Manful, 2010). Increased demand for high quality rice provides a better opportunity for rice growing countries to reduce rice imports and generate higher export revenues (Balasubramanian et al., 2007).

However, despite efforts to encourage local rice production by the rice sector in SSA, production is still low and unable to meet the growing demand for high quality rice. Thus, SSA has become increasingly dependent on rice imports (Seck et al., 2010; Demont, 2013). In addition, locally cultivated rice cultivars do not match imported rice in end-user qualities and hence there is a high preference for imported rice which often attracts higher premium prices in the market (Yi et al., 2009). Whereas the focus for most rice breeding programmes in SSA has been on increasing yield and enhancing stress tolerance (Manful, 2010), consumer preferences that are directly associated with grain cooking and eating quality traits (Oko et al., 2012) and considered a major driver of widespread variety uptake (Calingacion et al., 2015) are rarely incorporated during cultivar development.

Rice grain quality traits are important to all stakeholders in the rice value-chain including producers, millers and consumers (Koutroubas et al., 2004; Fitzgerald et al., 2009) and often depicts the nutritional and market value of rice. Furthermore, the choice of grain quality may be objective or subjective to the end-user but often the most important grain quality attributes common to all end-users include grain appearance, milling quality, cooking and eating quality (taste, tenderness), aroma/fragrance and nutritional quality (Lou et al., 2009). Among the aforementioned, grain cooking and eating quality together with aroma are most crucial grain quality traits that determine the premium price of rice (Wang et al., 2007; Sakthivel et al., 2009; Asghar et al., 2012; Demont, 2013). Among the key physicochemical properties affecting eating and cooking quality, amylose content (AC) is the most important (Muhammad, 2009; Yi et al., 2009).

Several methods have been established for determining AC including iodine binding (Juliano, 1971), near infrared spectroscopy (Wesley et al., 2003), size-exclusion chromatography (Ward et al., 2006) and asymmetric field-flow fractionation (Chiaramonte et al., 2012). Nonetheless, most of these methods require the use of expensive equipment such as spectrophotometer, auto-analyser, amylogram, near infrared instrument, and thermocycler that are not readily available in developing countries, because their acquirement and maintenance costs are often very expensive (Avaro et al., 2009; Caffagni et al., 2013). Avaro et al. (2009) developed a simple and low-cost method of classifying AC that uses a colour chart instead of a spectrophotometer, while Duldulao et al. (2012) suggested a modified rapid AC test.

The use of genetic markers in marker-assisted selection and genomic selection has provided further options for improving selection strategies (Akhtar et al., 2010) for complex traits which could complement conventional breeding methods (Biselli et al., 2014). In this regard, advances in genetic studies have developed molecular techniques that facilitate marker-assisted breeding (MAB) for improved cooking and eating qualities in rice (Phing et al., 2016). However, given that rice cooking and eating qualities are quantitative traits and thus subject to the control of several chromosomes and environmental effects (Zheng et al., 2008), improving the precision of quantitative trait loci (QTL) identification, mapping and association studies for these traits (Phing et al., 2016) will be critical in order to realize the full potential of MAB.

1.2 Taxonomy and origin of rice

Rice belongs to the genus, *Oryza,* which is one of the 12 genera within the tribe Oryzeae of the *Poaceae* family distributed in the tropical and temperate regions of the world (Vaughan et al., 2004). Two cultivated species of rice; the Asian rice (*Oryza sativa* L.) and African rice (*O. glaberrima* Steud) belong to genomic group AA (2n=24) of the genus *Oryza*, family Poacae and tribe Oryzeae. The genus *Oryza* has 22 wild species (either 2n=24 or 2n=48) that represent 10 genomic types (Vaughan et al., 2004). Within *O. sativa* there are three subspecies; *indica* Kato, *japonica* Kato and *javanica* (Roschevicz, 1931). The subspecies *japonica* has two strains, namely *tropical* and *temperate*. *Oryza sativa* is grown worldwide and *O. glaberrima* is grown mainly in West Africa (Jones et al., 1997). Furthermore, the rice cultivars can be grouped into three ecological varieties: *Indica* which are of tropical and sub-tropical distribution, *Javanica* which grow in Indonesia and *Japonica* of temperate distribution (Vaughan et al., 2004). The *Indica* varieties are long-grained rice, *Javanica* are broad-grained and *Japonica* is short-grained (Vaughan et al., 2004). The rice cultivars can be distinguished based on several agronomic and morphological traits such as; adaptation to different water regimes, growth habit and height, shapes, size and colour of the culm, leaf blade, panicle, hull and grain, and degree of pubescence. Additionally, rice cultivars can be categorized according to their physicochemical and grain quality properties that determine the nutritional and commercial value of the grains.

1.3 Rice grain quality

Grain quality is a complex character composed of many components such as nutrition quality, grain appearance and cooking and eating quality among others. Thus, preferences for quality may vary from one region to another (Nzomoi and Anderson, 2013). Very often, although the quality characteristics desired by the farmer, miller and consumer may be the same, each may place different emphasis on various quality characteristics (Khush, 2000). For example, the miller may be interested on total grain recovery and the proportion of head and broken rice on milling, whereas the farmer may emphasize on high yields and early maturing varieties, whilst the consumers may be more concerned with grain appearance, grain size and shape, cooking behaviour, taste, tenderness and aroma of cooked rice. In general, cooking and eating quality traits together with aroma or fragrance are most crucial grain quality traits that determine the premium price of rice (Wang et al., 2007; Sakthivel et al., 2009; Asghar et al., 2012; Demont, 2013). Furthermore, according to Muhammad (2009), high quality rice is often characterised by intermediate amylose content, intermediate gelatinisation temperature, soft gel consistency, strong aroma and a high degree of grain elongation.

1.3.1 Eating and cooking quality of rice

A major challenge in rice production is grain quality which stems from poor cooking and eating quality for most widely grown varieties (Oko et al., 2012). The eating and cooking qualities of rice are important in determining its commercial value and consumer acceptance (Phing et al., 2016). Rice contains a large amount of starch which digests more quickly than any other high starch food, making it highly distinctive among other cereal crops (Asghar et al., 2012). Grain appearance, processing, and cooking and taste qualities are directly related to three chemical properties of rice grain starch, namely; the amylose content (AC) (Juliano, 1971), gel consistency (GC) (Cagampang et al., 1973) and gelatinisation temperature (GT) (Little et al., 1958; Muhammad, 2009; Yi et al., 2009).

1.3.1.1 Amylose content

Amylose content (AC) of rice is considered to be one of the most important indirect indices of rice cooking and processing quality (Ni et al., 2011; Asghar et al., 2012) since it determines the hardness of cooked rice, gloss of the final product and rice-water ratio. Waxy or glutinous rice with highly reduced levels of amylose, does not expand in volume, is sticky and remains firm when cooked (Asghar et al., 2012). In contrast, the non-waxy or non-glutinous rice characterised by intermediate amylose, cooks moist and tender and does not become hard upon cooking, and hence more preferred (Muhammad, 2009). These differences clearly indicate the importance of amylose content as a selection criterion in rice (Juliano, 1971; Khush, 2000).

Amylose is synthesized by the granule-bound starch synthase (GBSSI) also known as waxy protein (Smith et al., 1997). GBSSI is encoded by the Waxy (Wx) locus (Umemoto et al., 2002) and the level of grain amylose is directly associated to the amount of GBSSI in the endosperm (Wang et al., 1995). Bao et al. (2008) also confirmed the close association of the Wx locus with amylose content. The Waxy gene is located on chromosome 6 and consists of 13 exons and 12 introns (Biselli et al., 2014). Two wild type alleles, Wx^a, primarily found in *indica* subspecies, and Wx^b, mainly found in *japonica* subspecies, have been found to predominate at the waxy locus for high and low AC respectively (Dobo et al., 2010). The difference between the two alleles is related to the presence in Wx^b of a G to T Single Nucleotide Polymorphism (SNP) at the 5′ splice site of the first 1,124 bp long intron (Biselli et al., 2014). Most of the waxy and low AC rice cultivars tend to express this polymorphism which results in the reduction of pre-mRNA splicing efficiency and promotion of alternative splicing at cryptic sites in exon 1 (Wang et al., 1995). This further contributes to a decrease in the production of functional enzymes, thereby causing glutinous and low amylose phenotypes (Ayres et al., 1997). Other reports suggest that AC is under the control of other loci in addition to the waxy locus though majority of these loci have largely remained unidentified (He et al., 1999; Aluko et al., 2004; Wambugu et al., 2017). Thus, the identification of additional potential candidate genes remains an important area of research focus with new targets for AC modification.

1.3.1.2 Gel consistency

Gel consistency (GC) is a good index of cooked rice texture. It measures the tendency of the cooked rice to harden after cooling (Muhammad, 2009). Different rice varieties may have similar amylose contents and physical dimensions such as grain shape, size and appearance, making it difficult to distinguish them accurately (Perez and Juliano, 1979). Thus, gel consistency would be most appropriate in discriminating such rice varieties (Muhammad, 2009).

Gel consistency may be defined as a measure of the flow characteristics of milled rice gel (100 mg) in 2 ml of 0.2 N KOH and is indexed by the length of the horizontal gel in mm in a 13 x 100 ml test tube (Muhammad, 2009). This test separates rice into hard (length 36 mm or less), intermediate (length 36-50 mm) and soft (length over 50 mm) gel consistency. Methods described by Little et al. (1958) can be used for determining gel consistency. Generally, intermediate amylose rice varieties have softer gel consistency and are more preferred for their tenderness (Muhammad, 2009; Phing et al., 2016). Hence, breeders tend to select for soft GC to improve the eating and cooking qualities in rice.

GC is reportedly controlled by the Waxy (Wx) gene that encodes granule-bound starch synthase (GBSS) on chromosome 6 (Fan et al., 2005; Li et al., 2007; Wang et al., 2007; Zhang et al., 2012). Lanceras et al. (2000) and Li et al. (2007) suggested that a tight linkage or pleiotropy between AC and GC is caused by the close proximity of their QTL positions. However, He et al. (1999) found two QTLs on chromosomes 2 and 7 for GC instead of on the Wx locus in doubled-haploid (DH) populations consisting of 132 pure lines. Minor QTLs for GC were also discovered by Lanceras et al. (2000) on chromosomes 6 and 7. Tian et al. (2005) also reported another minor QTL on chromosomes 1 and 2 in the DH population from their study. Gao et al. (2011) showed that the alkali degeneration locus (alk) acts as a modifier gene that controls GC in a negative fashion, supporting results by Wang et al. (2007) in which the alk locus has a minor effect on the GC. Su et al. (2011) conducted map-based cloning for a major QTL, namely, qGC-6. Their result further supported the idea that the Wx gene controls not only AC but also GC, and therefore it has an important role in improving both traits (Phing et al., 2016). Furthermore, Lanceras et al. (2000) concluded that GC was under the control of a single gene of major effect along with several modifier genes.

1.3.1.3 Gelatinization temperature

Generally, rice takes 14-21 minutes for cooking in already vigorously boiling water, while the time required for cooking has a positive correlation with gelatinization temperature (GT) (Khush, 2000). The GT is a physical property of starch and may be defined as the temperature range at which the starch granules swell irreversibly in hot water and starch crystalline structures begin to melt (Fitzgerald et al., 2009). Overall, GT ranges between 55^{o}C and 79^{o}C. This factor also classifies rice varieties as low (55^{o}C-69^{o}C), intermediate (70^{o}C-74^{o}C) and high (74.5^{o}C-79^{o}C) GT (Khush, 2000). Rice grains with intermediate GT are generally most preferred (Muhammad, 2009) and can be indirectly estimated by the degree of alkaline dispersion using the alkali spreading value (ASV) technique developed by Little et al. (1958).

The genetic basis of GT has been widely studied with reports showing that GT is controlled by the Waxy (Wx) gene that encodes granule-bound starch synthase (GBSS) on chromosome 6 (Tan et al., 1999; Li et al., 2007). Zhang et al. (2012) also showed that the Wx gene has a minor effect on the expression of GT. Contrary results for the gene that controls GT have also been found. Tian et al. (2005) showed that GT is independent of the Wx gene; they reported a major QTL corresponding to the alkali degeneration locus (alk) on chromosome 6. Also, according to He et al. (1999) and Fan et al. (2005), the alk locus has a major effect on the GT. In addition, Umemoto et al. (2002) showed that the starch synthase IIa (SSIIa) gene that encodes starch synthase IIa (SSIIa) is located on the alk locus of chromosome 6. Umemoto et al. (2004) further indicated that SSIIa is the enzyme that contributes to natural variation in GT. Also, association mapping studies by Xu et al. (2013) showed that the starch properties of waxy rice were primarily controlled by SSIIa and SSI. However, Shu et al. (2006) reported that another gene contributes to the GT variation in terms of ASV, namely, the alk2(t) gene. Shu et al. (2006) also suggested that the alk2(t) gene, which is 3.93 cm away from the

Wx gene, has two alleles responsible for low and high GT. To date, several functional SNPs have been reported to be associated with rice GT (Umemoto et al., 2002, 2004; Waters et al., 2006), with a view of identifying closely linked markers for improving the target trait.

1.3.1.4 Aroma in rice

Very often, aromatic or fragrant rice commands a higher price in the rice market than the non-aromatic rice varieties (Khush, 2000) because of their pleasant aroma, flavour and texture. Aromatic cultivars commonly sold in world trade include the Basmati rice of India and Pakistan and the Jasmine rice of Thailand. Aromatic rice is highly valued throughout Asia with wider acceptance in Europe, Australia, USA and the Middle East (Suwannaporn and Linnemann, 2008; Sakthivel et al., 2009). The chemistry of flavour in rice grain reveals the existence of numerous volatiles in fragrant rice although the relationships among them are not well established except for a major aromatic compound, 2-acetyl 1-pyrroline (2AP) (Jewel et al., 2011). Several studies have suggested 2AP as a principal aroma compound and badh2 as the candidate gene for fragrance (Kibria et al., 2008; Sakthivel et al., 2009). Weber et al. (2000), further suggests that over 300 aromatic rice varieties have been identified despite the low utilisation and production of these varieties. Given the economic and cultural importance of aromatic varieties, there is a need to further enhance their productivity whilst enhancing other important grain quality attributes.

1.3.2 Rice grain quality evaluation procedures

The genetic complexity of grain quality traits such as eating and cooking quality and the difficulty in accurate evaluation of these traits in early breeding generations, has constrained the development of rice varieties with superior eating and cooking qualities (Lestari et al., 2009). Some key physicochemical properties affecting the eating and cooking quality are amylose content (AC), pasting properties (PP), gel consistency (GC), gelatinization temperature (GT), and protein content (PC). Among the aforementioned, AC is the major determinant of rice processing and cooking and eating qualities (Muhammad, 2009; Yi et al., 2009). Methods have been established for determining the amylose content (Juliano, 1971), gel consistency (Cagampang et al., 1973) and gelatinisation temperature (Little et al., 1958). A simple and low-cost method for classifying AC in rice based on Juliano's method (1971), with the use of a low-cost colour chart instead of a spectrophotometer has also been developed (Avaro et al., 2009).

However, the need for highly accurate results has led to the use of most recent technologies for determining AC in rice. Subsequently, most of the AC quantification methods require the use of expensive equipment that are often not available in developing countries (Avaro et al., 2009). Thus, the use of marker-assisted selection (MAS) could overcome the inadequacies of AC measurements, given its low-cost and high throughput (Biselli et al., 2014). Furthermore, MAS can be applied as a selection tool in the early phases of a breeding programme (Borba et al., 2010), whereas AC direct analysis would require seed setting at harvest. In addition, DNA markers for AC are able to distinguish between homozygous and heterozygous parents and further provide a more absolute way of classifying Granule-Bound Starch Synthase (GBSS) alleles compared to AC assays since it avoids the impediments of modifier genes, cytoplasmic factors, and environmental effects (Biselli et al., 2014).

1.3.3 Genetic and molecular basis of eating and cooking quality in rice

The genetics of cooking and eating quality in rice has been widely reported (He et al., 1999; Li et al., 2003; Zhou et al., 2003). Amylose content has been found to be the major determinant of rice cooking and eating quality (Dobo et al., 2010; Biselli et al., 2014) and the Waxy (Wx) gene that encodes granule-bound starch synthase (GBSS) on chromosome 6 is critical for amylose synthesis (Biselli et al., 2014). Additionally, quantitative trait locus (QTL) analysis has shown that the Wx gene region controls the three major eating and cooking quality traits (AC, GC and GT) in rice (Tan et al., 1999; Lanceras et al., 2000; Li et al., 2003; Tian et al., 2005). The inheritance of rice cooking quality has also been widely studied (Pooni et al., 1992; Xu et al., 1995; Shi et al., 1997; Lin et al., 2005). Pooni et al. (1992) suggested that amylose was related to the effects of the maternal plant or cytoplasm, whereas Xu et al. (1995) reported that rice amylose content was mainly controlled by the triploid endosperm genotype without any cytoplasmic effect. Shi et al. (1997) indicated that direct seed effects, maternal effects and cytoplasmic effects were the main factors controlling amylose content, alkali spreading score and gel consistency respectively. Lin et al. (2005) reported that the AC of *japonica* rice was not only influenced by the genetic main effects from endosperm, cytoplasm and maternal plant genes but was also affected by genotype x environment (GE) interaction effects.

Furthermore, it has also been shown that other factors other than AC may be involved in regulating rice cooking and eating quality, since rice varieties with similar ACs have been observed to have different eating and cooking qualities (Liu et al., 2010). Liu et al. (2010) suggested the existence of other gene(s) that are non-allelic to the Wx gene that were mapped on chromosome 8 QTL cluster between flanking markers RM4955–RM8264 and G1149–R727. Other studies have confirmed marker associations with the QTLs for AC (Swamy et al., 2012; Tabkhkar et al., 2012), while Pandey et al. (2012) further suggested that the identified molecular markers could only differentiate between low AC varieties and high or intermediate AC varieties but not between varieties with intermediate and high AC. In another study, Yacouba et al. (2013) indicated that both AC and GC were not influenced by the *Wx* gene region. Furthermore, two QTLs were identified for AC; one QTL was detected at the interval of RM402-RM5963 on chromosome 6 corresponding to the *Alk* locus, and another QTL was detected on chromosome 8. These QTLs accounted for 8.6% and 5.7% of the total phenotypic variation respectively (Yacouba et al., 2013). Kottearachchi et al. (2014), used SSR markers RM 190 and RM 314, previously linked to AC and GT (Fan et al., 2005; Tabkhkar et al., 2012) for PCR amplification and identified SSR marker RM 190 as a potential marker in differentiating between low AC rice varieties over the intermediate and high AC. In another study to investigate the relationship between GBSS and AC in the US and European rice germplasm, Dobo et al. (2010) identified three single-nucleotide polymorphisms (SNP) in exons 1, 6 and 10, that differentiated between low (15-20%), intermediate (21-24%) and high AC (>25%).

Additionally, to provide a better discrimination between cultivars with AC greater than 25% from those with lower levels, Biselli et al. (2014) identified new SNPs by re-sequencing of the Waxy gene and 1kbp of the upstream region. Thus, given these developments, several markers and QTLs affecting rice quality traits have been identified and mapped in various mapping populations (Xing and Zhang, 2010), in order to apply marker-assisted selection for enhancing breeding efficiency. However, the identified QTLs may not be sufficient to elucidate the genetic basis of rice cooking and eating quality traits. Also, the varied nature of rice cooking and eating quality traits (Liu et al., 2010) underscores the need for identifying novel QTLs in order to design a breeding strategy for their improvement. In addition, Wang et al. (2010) suggested that knowledge of AC and its associated traits represents a major criterion in developing rice cultivars with desirable cooking and eating quality. Consequently, with the accumulated information on QTLs for cooking and eating quality traits and their closely linked markers (Sattari et al., 2015), QTL pyramiding can be applied as a direct approach for improving the target traits.

1.3.4 Genetic and molecular basis of grain yield in rice

Grain yield is a complex trait and is determined mainly by three component traits in rice, namely; number of panicles per plant, number of grains per panicle, and grain weight (Xing and Zhang, 2010). The number of panicles depends on the ability of the plant to produce primary, secondary, and tertiary tillers, while the number of grains per panicle largely depends on the number of spikelets and seed setting rate of the spikelets (Tripathi et al., 2012). In addition, grain weight is largely determined by grain size and the degree of filling (Xing and Zhang, 2010). A great difference in the levels of grain yield is found among different rice genotypes, with immense variability in the combinations of component traits (Tripathi et al., 2012). In addition, yield levels of rice varieties can also be greatly influenced by the prevailing environmental conditions and field management practices (Xing and Zhang, 2010).

Rice yield is considered a quantitative trait and is controlled by multiple genes, each contributing a small but significant effect (Tripathi et al., 2012). The trait is also influenced by the environment, which makes it more difficult to investigate (Xing and Zhang, 2010). Nevertheless, with the development of molecular markers, genome mapping, and QTL analysis technologies, the genetic basis of quantitative traits has been widely studied leading to the identification of several QTLs for yield-related traits (McCouch et al., 1988; Kurata et al., 1994; McCouch et al., 2002). Thus, the development of techniques for QTL validation and analysis has further contributed in deciphering the genetic basis of yield-related traits (Tripathi et al., 2012). Furthermore, efforts in rice functional genomics have led to the identification of several genes linked to yield-related QTLs (Miura et al., 2011). Given this background knowledge, the molecular basis of grain yield in rice can be exploited in yield improvement programmes through gene pyramiding. However, given the complex nature of grain yield, not a large number of genes can be simultaneously engineered. Hence, it is essential to select a few genes which can function synergistically in order to get the desired outcome (Tripathi et al., 2012). Thus, to achieve this objective, there is a need to extensively assess the expression patterns of the genes regulating yield-related traits in rice.

1.4 Breeding for improved yield and grain quality in rice

1.4.1 Role of farmer preferences and end-user grain qualities in rice

For most rice breeding programmes in Africa, the focus has been on increasing yield and enhancing stress tolerance much to the disadvantage of grain eating and cooking qualities (Manful, 2010). A great number of the varieties developed are often released after field testing without taking account of their grain quality attributes. Such varieties may satisfy the farmers' preferences but not the end-user qualities, thus the locally produced rice is unable to compete favourably with imported rice that has superior cooking and eating qualities. Rice grain quality is important to those involved in rice production, processing and consumption and affects the nutritional and commercial value of the grains. Thus, rice grain quality attributes may include; processing quality, appearance, nutrition, and cooking and taste qualities. Among these, consumers' choices of rice varieties are largely based on grain cooking and eating qualities (Oko et al., 2012).

Although preferences may vary from one group of consumers to another, rice grains with a pleasant fragrance and a soft texture usually achieve higher prices in national and international markets (Yi et al., 2009). For example, in Uganda, consumers prefer aromatic to non-aromatic rice, unbroken to broken, bulging after cooking to rice that does not bulge and white milled rice to brown rice (MAAIF, 2012). In addition, a study by Lamo (2010) in Uganda reported that overall, high yield potential, high market value and aroma were the most important choice of preferences in a rice variety by farmers. Further, in another study by Mzengeza (2010) in Malawi, farmers identified large grain size, aroma and rice grain that tends to remain separate after cooking as most preferred traits in a rice variety. In Tanzania, a study by Kashenge (2010) reported that yield potential, aroma and early maturity were the most preferred traits in a rice variety. Furthermore, Tanzania, the second largest producer of rice in East Africa, has not only sustained its domestic market for rice but also exports substantial quantities of high-quality fragrant rice to its neighbouring countries at a considerable premium over imported rice (Demont, 2013). Therefore, for effective breeding, farmer and end-user variety preferences have become critical for most crop improvement programmes, while the integration of participatory plant breeding (PPB) approaches has been useful in eliciting farmers' preferred varieties and encouraging wider uptake of new varieties (Ceccarelli, 2012; Ceccarelli et al., 2012).

1.4.2 Genetic diversity for yield-related and grain quality traits

The study of genetic diversity in rice is a critical component of plant genetics, breeding, conservation and in tracing the evolution history and pedigree of a rice variety (Tang et al., 2016). Breeding for improved grain yield and quality requires the selection of parents with a wider genetic diversity to ensure genetic gains (Lapitan et al., 2007). Thus, sufficient knowledge about genetic diversity in the genepool is a prerequisite to adopt an efficient and valuable breeding approach (Lapitan et al., 2007). In addition, characterization of rice germplasm is essential to provide information on different agro-morphologically important traits carried by each genotype to ensure maximum utilization of the germplasm collection by the end-users (Ayres et al., 1997).

Several strategies have been adopted for characterization of yield and grain quality traits in rice including, morphological (Devi et al., 2016) and molecular markers (Lapitan et al., 2007). The use of molecular markers for assessing the genetic variability and relatedness among crop germplasm is a valid improved approach (Vithyashini and Wickramasinghe, 2016) compared to the use of morphological markers. Morphological markers are limited in number and are often influenced by the environment and hence unreliable (Oloka et al., 2015). Microsatellite loci, also known as simple sequence repeats (SSRs) are among the most commonly used molecular markers since they are abundant, codominant and interspersed throughout the genome (Lapitan et al., 2007). However, the high demand for low cost sequence data has driven the development of high-throughput sequencing platforms or next-generation sequencing (NGS) technologies which has facilitated the large-scale discovery of single nucleotide polymorphisms (SNPs) in various plant species (Tang et al., 2016). Consequently, SNPs are rapidly replacing SSR markers because they are more abundant, stable, amenable to automation, efficient, and increasingly cost-effective (McCouch et al., 2010). Furthermore, the effectiveness of genotyping-by-sequencing (GBS), an NGS based method that takes advantage of reduced representation to enable high-throughput genotyping of a large number of SNP markers (Tang et al., 2016), has not been well exploited in rice. Thus GBS forms an important research focus for SNP genotyping in rice to further guide breeding efforts for rice grain quality and yield improvement.

1.4.3 Selection for grain yield and quality based on their attributing factors

The agronomic value of rice is depicted by increased grain yield and its attributing factors such as number of panicles per plant, number of grains per panicle and weight of 1000 grains (Saha et al., 2016). A common weakness among most cultivars with superior grain quality is low yield and consequently rice breeders are focussing on improving agronomic traits to gain better grain yield. Given that grain yield is controlled by a combined effect of various traits, selection of parents on the basis of yield alone can be misleading (Rashid et al., 2014). In addition, the success of a breeding programme depends on the amount of genetic variability present in the population and the extent to which the desirable traits are heritable (Devi et al., 2016). Thus, an efficient selection strategy requires knowledge about relationship between yield and its contributing characters.

Furthermore, the study of relationships among quantitative traits is important for assessing the feasibility of joint selection for two or more traits where correlation analysis of characters can be used as a tool for indirect selection (Saha et al., 2016). However, correlation analysis between grain yield and its attributing characters may be misrepresentative due to an over estimation or underestimation of its association with other characters. Therefore, splitting of total correlation into direct and indirect effects further provides a more meaningful interpretation of plausible associations, where path coefficient is useful in specifying the cause and effect relationship and assessing the relative importance of each variable (Rai et al., 2015). A study by Zahid et al. (2006) in Basmati rice indicated that number of grains per panicle had the highest positive correlation with grain yield and further suggested that this character had a positive direct effect on grain yield. In another study, Premkumar et al. (2016) proposed that genetic improvement of grain quality and yield can be achieved by selecting for grain quality characters having high positive correlation and positive direct effect on grain yield. Thus, the use of correlation in combination with path coefficient analysis can be an important tool for identifying associations between characters and in quantifying the relative importance of each character.

In addition, principal component and factor analysis may be used in modelling complex traits such as yield and grain quality in rice. The principal component analysis (PCA) is a multivariate statistical method for exploring and simplifying complex data sets, where, each principal component is a linear combination of the original variables, and therefore it is often possible to ascribe the meaning to what the components represent (Lewis and Lisle, 1998). The PCA generates the total variance of variables, it describes the maximum variance within a data set and is a function of primary traits (Mohsen et al., 2014), which makes this approach more effective in deciding which agronomic traits contribute most to yield or grain quality.

1.4.4 Combining ability analysis for yield and grain quality traits

The success of any plant breeding programme relies on the choice of appropriate genotypes as parents in a hybridization programme such that combining ability studies of the parents will provide guidance in the selection of better parents for effective breeding. In addition, combining ability analysis provides information on additive and dominance variance and that is important in choosing parents, crosses and appropriate breeding procedure to be followed in selecting for desirable segregants (Thakare et al., 2010). Consequently, breeding strategies based on hybrid production depends on a high degree of heterosis as well as the specific combining ability (SCA) of crosses.

Furthermore, diallel analysis is one of the most important tools for estimating the general combining ability (GCA) of parents and selection of desirable parents and crosses with high SCA for exploiting heterosis (Rahimi et al., 2010). Srivastava (2000) suggested that heterosis breeding can effectively contribute to yield enhancement by 30% to 400% as well as successfully be used in the improvement of other desirable quantitative and qualitative traits in crops. Combining ability studies for yield and grain quality traits in rice have been described (Saleem et al., 2010; Thakare et al., 2010; Roy et al., 2012) with the view of identifying good combiners useful for developing populations with favourable genes for yield and quality traits in rice.

1.4.5 Genotype-by-environment interaction effects for rice grain yield and quality

Multi-environment trials (MET) have been useful in evaluating yield stability performance of genetic materials under variable environmental conditions (Farshadfar et al., 2012), such that a genotype grown in different environments will often show substantial fluctuations in yield performance. Such changes can be influenced by variable environmental conditions referred to as genotype-by-environment (GE) interaction (Sharifi et al., 2009; Sharifi et al., 2010). However, GE interaction lessens genetic progress in breeding programmes by decreasing the association between phenotypic and genotypic values (Mohamed, 2013). Therefore, GE interaction can either be exploited by selecting superior genotypes for each specific target environment or avoided by selecting widely adapted and stable genotypes across wide range of environments (Ceccarelli, 1989).

Rice eating qualities are affected by environmental conditions such as weather, soil texture, biotic and abiotic stresses and cultural management (Sharifi et al., 2010). The existence of GE interaction for cooking quality traits of rice has been reported (Gravois and Webb, 1997; Shi et al., 1997; Chen and Zhu, 1999; Chen and Zhu, 2002; Lin et al., 2005). Shi et al. (1997) suggested that AC and GT were mainly controlled by genetic effects with the presence of GE interaction effects. Gravois and Webb (1997) showed that general combining ability (GCA) x year was significant for all of the amylograph viscosity characteristics, while Chen and Zhu (2002) revealed that GE interactions were expressed mainly as dominance x environment and cytoplasm x environment interaction for AC, GC and GT.

Furthermore, in a study by Jin et al. (2005) on the effects of temperature on grain-filling revealed that high temperature at the grain-filling stage decreased AC of rice. Therefore, understanding gene expression in different environments is necessary for improving rice eating and cooking quality traits (Sharifi et al., 2010).

1.4.6 Conventional breeding for eating and cooking quality in rice

There has been considerable success in improving rice for eating and cooking quality through several conventional rice breeding approaches such as backcrossing and pedigree breeding. However, users of conventional breeding techniques have encountered several challenges in improving cooking and eating quality traits in rice (Phing et al., 2016). Jennings et al. (1979) reported that conventional backcrossing is most appropriate for selecting traits that are controlled by a single gene. Nevertheless, given the quantitative nature of grain quality traits (Yano and Sasaki, 1997) coupled with the triploid endospermic nature of the genotypes and interactions between genotypes and the environment, improving eating and cooking quality through conventional backcrossing is complex. Furthermore, the use of pedigree breeding method for improving rice eating and cooking qualities is time-consuming (Jennings et al., 1979) and requires seed setting at harvest for laboratory evaluation. Given the foregoing, conventional rice breeding approaches have been less efficient in selection of high-quality rice. The use of molecular marker technology provides a more reliable option for selection of complex traits and thus more useful in complementing conventional breeding efforts.

1.4.7 Marker-assisted breeding for improving eating and cooking qualities

Improving rice grain yield and quality are important considerations (Borba et al., 2010), but remain an unprecedented challenge for most rice breeding programmes (Wang et al., 2012). Knowledge of the nature and magnitude of the genetic variation governing the inheritance of grain quality and yield-related traits is essential for effective genetic improvement (Dhanwani et al., 2013). Conventional breeding methods have resulted in limited success in the development of high quality new cultivars due to the quantitative nature of grain quality traits and environmental variations (Yi et al., 2009; Wang et al., 2012). Molecular marker technology provides options for improving selection strategies (Akhtar et al., 2010), and facilitates the selection of complex traits during the breeding process because they are reliable and unaffected by environmental conditions (Yi et al., 2009). Marker assisted selection (MAS) has also been useful for genotyping of accessions at an early stage for traits that are normally evaluated after harvesting (Borba et al., 2010). A number of quantitative trait loci (QTLs) affecting rice yield and quality traits have been identified and mapped for different populations (Xing and Zhang, 2010). However, the presence of epistasis and QTL x environment interaction makes it difficult to apply MAS for genetic improvement of complex traits (Xu and Crouch, 2008).

Additionally, the formation of QTL clusters due to a single gene with pleiotropic effects on multiple traits or due to the ‘‘linkage drag’’ of multiple linked genes, each affecting a different trait limits breeding efforts (Yamamoto et al., 2009). Nevertheless, efforts in QTL mapping and validation has not only improved the use of MAS for transfer of desirable QTL clusters and unlocking of undesirable linkage, but also provided insight into the genetic mechanisms regulating related traits (Wang et al., 2012). Yi et al. (2009), successfully improved fragrance and intermediate AC in a Myanmar rice cultivar (Manawthukha) by marker-assisted backcrossing (MABC). Other achievements in improving complex traits by MAS using advanced backcross populations, chromosomal segment substitution lines, near-isogenic lines (NIL), and heterogeneous inbred families (HIF) with uniform genetic background have been reported (Xie et al., 2008; Maas et al., 2010; Tyagi et al., 2014). Thus, the use of DNA marker technology such as MABC can reliably be used for introgression of desirable rice grain quality traits in the background of high yielding rice cultivars.

1.4.8 Prospects of genomics-assisted breeding

Rice genome sequence data has simplified the identification and cloning of genes and QTLs for yield and grain quality traits and thus provided an important resource for detecting allelic variation and for genome assisted breeding programmes (Khush, 2013). Hence, desirable genes can be pyramided in elite rice cultivars to enhance their yield potential and grain quality through MAS. Furthermore, development, identification and validation of functional SNP markers for target genes has enhanced gene pyramiding into elite germplasm through MAS (Xing and Zhang, 2010). Map-based cloning has resulted in isolation of several genes for resistance to biotic and abiotic stresses as well as yield related traits, which has further improved the possibility of applying MAS for yield enhancement (Khush, 2013). The high demand for low cost sequence data has driven the development of high-throughput next-generation sequencing (NGS) technologies that can generate millions of sequences simultaneously (He et al., 2014), while advances in genotyping-by-sequencing (GBS) offer a greatly simplified library production procedure more amenable to use on large numbers of individuals (Elshire et al., 2011). GBS is thus increasingly becoming an important cost-effective and unique tool for genomics-assisted breeding in a range of plant species (He et al., 2014) and hence undoubtedly an ultimate MAS tool to accelerate plant breeding and crop improvement.

In addition, with the advance in microarray-based marker technology, Diversity Arrays Technology (DArT) markers have become the genetic markers of choice for construction of high-density maps, QTL mapping and genetic diversity analysis based on their efficiency and low cost (Gupta et al., 2008). Additionally, by combining the complexity reduction of the DArT method with high-throughput NGS technologies, the DArT sequencing (DArTseq) platform was developed signifying a new implementation of sequencing of complexity reduced representations (Sánchez-Sevilla et al., 2015). Consequently, DArTseq markers based on GBS technology have been successfully applied for linkage mapping, QTL identification in bi-parental mapping populations, genome wide association studies (GWAS), genetic diversity, as well as in marker-assisted and genomic selection (Sánchez-Sevilla et al., 2015). Hence, DArTseq has been widely applied (Kilian et al., 2012; Courtois et al., 2013; Von Mark et al., 2013) and is rapidly gaining popularity as a preferred method of genotyping by sequencing (Sánchez-Sevilla et al., 2015). With these developments in genetic marker technologies, breeding efforts for complex traits has been made more efficient and cost-effective thus improving the use of MAS and genomic selection for enhancement of yield and grain quality traits in rice.

1.5 Conclusion and future outlook

Improving grain yield and quality are important considerations in a rice breeding programme not only for the widespread uptake of a new variety but also in enhancing the nutritional and commercial value of the rice grain. However, the complex nature of these traits makes improvement through breeding difficult especially for rice breeding programmes in SSA. Consequently, conventional breeding methods have had limited success in improving eating and cooking quality in rice, while accurate evaluation of these traits during the early phases of a breeding programme is a major challenge. Given the need for highly accurate results during quantification of eating and cooking quality traits, there has been an increased introduction of high-throughput technologies. Furthermore, the high demand for low cost sequence data has driven the development of high-throughput sequencing platforms or next-generation sequencing (NGS) technologies. Advances made in microarray-based marker technology have encouraged the use of Diversity Arrays Technology (DArT) markers for construction of high-density maps, QTL mapping and genetic diversity analysis based on their efficiency and low cost. In addition, by combining the complexity reduction of the DArT method with high-throughput NGS technologies, the DArTseq platform was developed to reduce on sequence redundancy. Furthermore, efforts in QTL mapping and validation have improved the use of MAS and genomic selection for genetic improvement of complex traits. More recently, rice genome sequence data has simplified the identification and cloning of genes and QTL for yield and grain quality traits, thus providing an important cost-effective and unique tool for genomics-assisted breeding and a critical MAS tool for accelerating plant breeding and crop improvement for grain yield and quality in rice.

References

Akhtar, S., M. Bhat, S.A. Wani, K. Bhat, S. Chalkoo, M. Mir and S.A. Wani. 2010. Marker Assisted Selection in Rice. Journal of Phytology. 2(10).

Asghar, S., F.M. Anjum, R.M. Amir and M.A. Khan. 2012. Cooking and eating characteristics or rice (*Oryza sativa* L.). Pakistan Journal of Food Sciences. 22(3): 128-132.

Avaro, M.R.A., T. Ly and T. Yoshida. 2009. A simple and low-cost method to classify amylose content of rice using a standard color chart. Plant Production Science 12: 97-99.

Ayres, N.M., A.M. McClung, P.D. Larkin, H.F.J. Bligh, C.A Jones and W.D Park. 1997 Microsatellites and a single-nucleotide polymorphism differentiate apparent amylase classes in an extended pedigree of US rice germplasm. Theoretical and Applied Genetics. 94:773–781.

Balasubramanian, V., M. Sie, R.J. Hijmans and K. Otsuka. 2007. Increasing Rice Production in Sub-Saharan Africa: Challenges and Opportunities. Advances in Agronomy. 94: 55-133.

Bao, J., L. Jin, P. Xiao, S. Shen, M. Sun and H. Corke, et al. 2008. Starch physicochemical properties and their associations with microsatellite alleles of starch-synthesizing genes in a rice RIL population. Journal of Agricultural and Food Chemistry, 56, 1589–94.

Biselli, C., D. Cavalluzzo, R. Perrini, A. Gianinetti, P. Bagnaresi, S. Urso, G. Orasen, F. Desiderio, et al. 2014. Improvement of marker-based predictability of Apparent Amylose Content in japonica rice through GBSSI allele mining. Rice. 7(1): 1.

Borba, T.C.d.O., R.P.V. Brondani, F. Breseghello, A.S.G. Coelho, J.A. Mendonça, P.H.N. Rangel and C. Brondani. 2010. Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.). Genetics and Molecular Biology. 33(3): 515-524.

Caffagni, A., G. Albertazzi, G. Gavina, S. Ravaglia, A. Gianinetti, N. Pecchioni and J. Milc. 2013. Characterization of an Italian rice germplasm collection with genetic markers useful for breeding to improve eating and cooking quality. Euphytica 194: 383-399.

Cagampang, G.B., C.M. Perez and B.O. Juliano. 1973. A gel consistency test for eating quality of rice. Journal of the Science of Food and Agriculture 24: 1589-1594.

Calingacion, M., L. Fang, L. Quiatchon-Baeza, R. Mumm, A. Riedel, R.D. Hall and M. Fitzgerald. 2015. Delving deeper into technological innovations to understand differences in rice quality. Rice 8: 6.

Ceccarelli, S. 1989. Wide adaptation: how wide? Euphytica 40: 197-205.

Ceccarelli, S. 2012. Plant breeding with farmers-technical manual. ICARDA, PO Box 5466, Aleppo, Syria.: xi + 126.URL:http://www.farmersrights.org/pdf/ TechManual_Ceccarelli.pdf.

Ceccarelli, S., A. Galiè, Y. Mustafa and S. Grando. 2012. Syria: Participatory barley breeding-farmers' input becomes everyone's gain. The Plant breeding with farmers-a technical manual 122: 53-66.

Chen, J. and J. Zhu. 1999. Genetic effects and genotype x environment interactions for cooking quality traits in Indica-Japonica crosses of rice (*Oryza sativa* L.). Euphytica 109: 9-15.

Chen, J. and J. Zhu. 2002. Genetics effects for cooking quality characters in Indica-Japonica crosses of rice. Journal of Biomathematics 17: 226-234.

Chiaramonte, E., L. Rhazi, T. Aussenac and D.R. White Jr. 2012. Amylose and amylopectin in starch by asymmetric flow field-flow fractionation with multi-angle light scattering and refractive index detection (AF4-MALS-RI). Journal of Cereal Science 56: 457-463.

Courtois, B., A. Audebert, A. Dardou, S. Roques, T. Ghneim-Herrera, G. Droc, J. Frouin, L. Rouan, et al. 2013. Genome-wide association mapping of root traits in a japonica rice panel. Plos One 8: e78037.

Demont, M. 2013. Reversing urban bias in African rice markets: A review of 19 National Rice Development Strategies. Global Food Security. 2(3): 172-181.

Devi, K.R., K. Parimala, V. Venkanna, N. Lingaiah, Y. Hari and B.S. Chandra. 2016. Estimation of Variability for Grain Yield and Quality Traits in Rice (*Oryza sativa* L.). International Journal of Pure and Applied Bioscience. 4(2): 250-255.

Dhanwani, R.K., A. Sarawgi, A. Solanki and J.K. Tiwari. 2013. Genetic variability analysis for various yield attributing and quality traits in rice (*Oryza sativa* L.). The Bioscan 8(4): 1403-1407.

Dobo, M., N. Ayres, G. Walker and W.D. Park. 2010. Polymorphism in the GBSS gene affects amylose content in US and European rice germplasm. Journal of Cereal Science 52: 450-456.

Duldulao, J., K. Bergonio and C. Estonilo. 2012. Philippine rice R & D Highlights. Rice Chemistry and Food Science Division.www.philrice.gov.ph/wp-content/uploads/2015/05/2013_RCFSD.pdf.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE. 6(5): e19379.

Fan, C., X. Yu, Y. Xing, C. Xu, L. Luo and Q. Zhang. 2005. The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. Theoretical and Applied Genetics. 110(8): 1445-1452.

Farshadfar, E., R. Mohammadi, M. Aghaee and Z. Vaisi. 2012. GGE biplot analysis of genotype x environment interaction in wheat-barley disomic addition lines. Australian Journal of Crop Science. 6(6): 1074.

Fitzgerald, M.A., S.R. McCouch and R.D. Hall. 2009. Not just a grain of rice: the quest for quality. Trends in Plant Science 14: 133-139.

Gao, Z., D. Zeng, F. Cheng, Z. Tian, L. Guo, Y. Su, et al. 2011. ALK, the key gene for gelatinization temperature, is a modifier gene for gel consistency in rice. Journal of Integrative Plant Biology. 53(9): pp.756-765.

Gravois, K. and B. Webb. 1997. Inheritance of long grain rice amylograph viscosity characteristics. Euphytica 97: 25-29.

Gupta, P., S. Rustgi and R. Mir. 2008. Array-based high-throughput DNA markers for crop improvement. Heredity 101: 5-18.

He, J., X. Zhao, A. Laroche, Z.-X. Lu, H. Liu and Z. Li. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in Plant Science. 5: 484.

He, P., S. Li, Q. Qian, Y. Ma, J. Li, W. Wang, Y. Chen and L. Zhu. 1999. Genetic analysis of rice grain quality. Theoretical and Applied Genetics. 98(3-4): 502-508.

Jennings, P., W. Coffman and H. Kauffman. 1979. Grain quality. Rice Improvement.: 101-120.

Jewel, Z.A., A.K. Patwary, S. Maniruzzaman, R. Barua and S.N. Begum. 2011. Physico-chemical and Genetic Analysis of Aromatic Rice (*Oryza sativa* L.) Germplasm. A Scientific Journal of Krishi Foundation. 9(1&2): 82-88.

Jones, M.P., M. Dingkuhn, M. Aluko and M. Semon. 1997. Interspecific *O. sativa* L. x *O. glaberrima,* Steud, progenitor in upland rice improvement. Euphytica 92: 237-246.

Juliano, B.O. 1971. A simplified assay for milled-rice amylose. Cereal Sci. Today 16: 334-340,360. URL http://ci.nii.ac.jp/naid/10013198955/en/.

Kashenge, K.S. 2010. Breeding investigations for salt tolerance in rice incorporating characterisation of salt affected soils and farmers perceptions and preferences for tolerant cultivars in North-Eastern Tanzania. University of KwaZulu-Natal, Pietermaritzburg.

Khush, G. 2000. Taxonomy and origin of rice. Aromatic Rices: 5-13.

Khush, G. 2013. Strategies for increasing the yield potential of cereals: case of rice as an example. Plant Breeding 132: 433-436.

Kibria, K., M. Islam and S. Begum. 2008. Screening of aromatic rice lines by phenotypic and molecular markers. Bangladesh Journal of Botany 37: 141-147.

Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia, H. Blois, V. Caig, K. Heller-Uszynska, et al. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. Data Production and Analysis in Population Genomics: Methods and Protocols: 67-89.

Kottearachchi, N., R. Peiris and S. Rebeira. 2014. Application of DNA markers for the detection of amylose content in Sri Lankan rice (*Oryza Sativa* L.) varieties. Asian Journal of Agricultural Biology. 2(1): 44-50.

Koutroubas, S.D., F. Mazzini, B. Pons and D.A. Ntanos. 2004. Grain quality variation and relationships with morpho-physiological traits in rice (*Oryza sativa* L.) genetic resources in Europe. Field Crops Research 86: 115-130.

Kurata, N., Y. Nagamura, K. Yamamoto, Y. Harushima and N. Sue. 1994. A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genetics. 8: 365-372.

Lamo, J. 2010. Genetic studies on drought tolerance and grain shattering in rice. University of KwaZulu-Natal, Pietermaritzburg.

Lanceras, J.C., Z.L. Huang, O. Naivikul, A. Vanavichit, V. Ruanjaichon and S. Tragoonrung. 2000. Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). DNA Research. 7(2): 93-101.

Lapitan, V.C., D.S. Brar, T. Abe and E.D. Redoña. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. Breeding Science. 57(4): 263-270.

Lestari, P., T.H. Ham, H.H. Lee, M.O. Woo, W. Jiang, S.H. Chu, S.-W. Kwon, K. Ma, et al. 2009. PCR marker-based evaluation of the eating quality of japonica rice (*Oryza sativa* L.). Journal of Agricultural and Food Chemistry. 57(7): 2754-2762.

Lewis, G. and T. Lisle. 1998. Towards better canola yield; a principal components analysis approach. Proceed 9th Australian Conference. Wagga Wagga. School of Land and Food. The University of the Queensland. Lawea, Qld (Vol. 4345).

Li, Z., J. Wan, J. Xia and M. Yano. 2003. Mapping of quantitative trait loci controlling physico-chemical properties of rice grains (*Oryza sativa* L.). Breeding Science. 53(3): 209-215.

Li, W., L. Zhang, Y. Cao, Y. Chen, L.G. Wang and B. Zhao. 2007. Correlation coefficients between Wx gene and rice edible quality of indica rice. Hubei Agricultural Sciences. 46:(6), p.864.

Lin, J., C. Shi, M. Wu and J. Wu. 2005. Analysis of genetic effects for cooking quality traits of japonica rice across environments. Plant Science 168(6): 1501-1506.

Little, R.R., G.B. Hilder and E.H. Dawson. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry. 35(2): 111-126. .

Liu, X., X. Wan, X. Ma and J. Wan. 2010. Dissecting the genetic basis for the effect of rice chalkiness, amylose content, protein content, and rapid viscosity analyzer profile characteristics on the eating quality of cooked rice using the chromosome segment substitution line population across eight environments. Genome 54: 64-80.

Lou, J., L. Chen, G. Yue, Q. Lou, H. Mei, L. Xiong and L. Luo. 2009. QTL mapping of grain quality traits in rice. Journal of Cereal Science 50: 145-151.

MAAIF. 2012. Ministry of Agriculture Animal Industry and Fisheries, Uganda National Rice Development Strategy (NRDS), 2008-2018. p. 8-12.

Maas, L.F., A. McClung and S. McCouch. 2010. Dissection of a QTL reveals an adaptive, interacting gene complex associated with transgressive variation for flowering time in rice. Theoretical and Applied Genetics. 120(5): 895-908.

Manful, J. 2010. Developing rice varieties with high grain quality in Africa: Challenges and prospects. Second Africa Rice Congress, Bamako, Mali, 22–26 March 2010: Innovation and Partnerships to Realize Africa's Rice Potential.

McCouch, S., G. Kochert, Z. Yu, Z. Wang and G. Khush. 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics. 76: 815-829.

McCouch, S., L. Teytelman, Y. Xu, K. Lobos and K. Clare. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Research. 9: 199-207.

McCouch, S.R., K. Zhao, M. Wright, C.W. Tung, K. Ebana, M. Thomson, et al. 2010. Development of genome-wide SNP assays for rice. Breeding Science. 60(5):524–35.

Miura K, M. Ashikari and M. Matsuoka. 2011. The role of QTLs in the breeding of high-yielding rice. Trends in Plant Science. 16:319–326

Mohamed, N.E. 2013. Genotype by environment interactions for grain yield in bread wheat (*Triticum aestivum* L.). Journal of Plant Breeding and Crop Science. 7(5): 150-157.

Mohsen, J., M. Zahra and S. Naser. 2014. Multivariate statistical analysis of some traits of bread wheat for breeding under rainfed conditions. Journal of Agricultural Sciences. 59(1): 1-14.

Muhammad, A. 2009. Aromatic Rices of Pakistan-a review. Pakistan Journal of Agricultural Research 22: 154-160.

Ni, D., S. Zhang, S. Chen, Y. Xu, L. Li, H. Li, Z. Wang, X. Cai, et al. 2011. Improving cooking and eating quality of Xieyou57, an elite indica hybrid rice, by marker-assisted selection of the Wx locus. Euphytica 179: 355-362.

Mzengeza, T. 2010. Genetic studies of grain and morphological traits in early generation crosses of Malawi rice (*Oryza* sativa L.) landraces and NERICA varieties. University of KwaZulu-Natal, Pietermaritzburg.

Nzomoi, J. and I. Anderson. 2013. The Rice Market in East Africa. Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists, September 22-25, 2013,Hammamet,Tunisia. p. 14.

Oko, A.O., B.E. Ubi and N. Dambaba. 2012. Rice Cooking Quality and Physico-Chemical Characteristics: a Comparative Analysis of Selected Local and Newly Introduced Rice Varieties in Ebonyi State, Nigeria. Food and Public Health 2: 43-49.

Oloka, B.M., J. Lamo, P. Rubaihayo, P. Gibson and J. Vorster. 2015. The use of multiplexed simple sequence repeat (SSR) markers for analysis of genetic diversity in African rice genotypes. African Journal of Biotechnology 14: 1533-1542.

Pandey, M.K., N.S. Rani, M.S. Madhav, R.M. Sundaram, G.S. Varaprasad, A.K.P. Sivaranjani, A. Bohra, G.R. Kumar, et al. 2012. Different isoforms of starch-synthesizing enzymes controlling amylose and amylopectin content in rice (*Oryza sativa* L.). Biotechnology Advances. 30(6): 1697-1706.

Perez, C.M. and B.O. Juliano. 1979. Indicators of eating quality for non-waxy rices. Food Chemistry, 4(3), pp.185-195.

Phing, W.C., M.A. Latif, M. Y. Rafii, M.R. Ismail and A. Puteh. 2016. Advances to improve the eating and cooking qualities of rice by marker-assisted breeding. Critical Reviews in Biotechnology. 36(1): 87-98.

Pooni, H., I. Kumar and G. Khush. 1992. A comprehensive model for disomically inherited metrical traits expressed in triploid tissues. Heredity 69: 166-174.

Premkumar, R., R. Gnanamalar and C. Anandakumar. 2016. Correlation and path coefficient analysis of grain quality traits in Rice (*Oryza sativa* L.). Indian Journal Of Agricultural Research. 50 (1): 27-32.

Rahimi, M., B. Rabiei, H. Samizadeh and A. Kafi Ghasemi. 2010. Combining ability and heterosis in rice (*Oryza sativa* L.) cultivars. Journal of Agricultural Science and Technology. 12: 223-231.

Rai, P., U. Sarker, P. Roy and A. Islam. 2015. Character association in F4 generation of rice (*Oryza sativa* L.). Bangladesh Journal of Plant Breeding and Genetics. 26(2): 39-44.

Rashid, K., I. Kahliq, M.O. Farooq and M.Z. Ahsan. 2014. Correlation and cluster analysis of some yield and yield related traits in Rice (*Oryza sativa* L.). Journal of Recent Advances in Agriculture. 2(6): 271-276.

Roschevicz, J.R. 1931. A contribution to the knowledge of rice. Bulletin of Applied Botany Genetics and Plant Breeding 27: 1-33.

Roy, S., A. Banerjee, B.K. Senapati and G. Sarkar. 2012. Comparative analysis of agro-morphology, grain quality and aroma traits of traditional and Basmati-type genotypes of rice, *Oryza sativa* L. Plant Breeding. 131(4): 486-492.

Rutsaert, P., M. Demont and W. Verbeke. 2011. Consumer preferences for rice in Africa. Realizing Africa's rice promise. CAB International. p. 218-229.

Saha, P.S., M.U. Islam, M.S. Islam and M.A. Salam. 2016. Analysis of Yield Components and Aroma of Small Grain Aromatic Rice (*Oryza sativa* L.) in Bangladesh. The Agriculturists. 13(2): 17-24.

Sakthivel, K., R.M. Sundaram, N. Shobha Rani, S.M. Balachandran and C.N. Neeraja. 2009. Genetic and molecular basis of fragrance in rice. Biotechnology Advances 27: 468-473.

Saleem, M.Y., J.I. Mirza and M.A. Haq. 2010. Combining ability analysis for yield and related traits in basmati rice (*Oryza sativa* L.). Pakistan Journal of Botany. 42(1): 627-637.

Sánchez-Sevilla, J.F., A. Horvath, M.A. Botella, A. Gaston, K. Folta, A. Kilian, B. Denoyes and I. Amaya. 2015. Diversity Arrays Technology (DArT) marker platforms for diversity analysis and linkage mapping in a complex crop, the octoploid cultivated strawberry (Fragaria× ananassa). Plos One 10: e0144960.

Sattari, A., N. Mahdinezhad, B. Fakheri, M. Noroozi, and H. Beheshtizadeh. 2015. Improvement of the eating and cooking qualities of rice: a review. International Journal of Farming and Allied Sciences.*4* (2), pp.153-160.

Seck, P.A., E. Tollens, M.C.S. Wopereis, A. Diagne and I. Bamba. 2010. Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. Food Policy 35: 403-411.

Sharifi, P., H. Dehghani, A. Moumeni and M. Moghaddam. 2010. Genetic main effect and genotype× environment interaction for cooking quality traits in a diallel set of Indica rice (*Oryza sativa* L.) varieties. Crop and Pasture Science. 61(6): 475-482.

Sharifi, P., H. Dehghani, A. Mumeni and M. Moghaddam. 2009. Genetic and Genotype × Environment Interaction Effects for Appearance Quality of Rice. Agricultural Sciences in China 8: 891-901.

Shi, C., J. Zhu, R. Zang and G. Chen. 1997. Genetic and heterosis analysis for cooking quality traits of indica rice in different environments. Theoretical and Applied Genetics. 95(1-2): 294-300.

Shu, X.L., S.Q. Shen, J.S. Bao, D.X. Wu, Y. Nakamura and Q.Y. Shu. 2006. Molecular and biochemical analysis of the gelatinization temperature characteristics of rice (*Oryza sativa* L.) starch granules. Journal of Cereal Science, 44(1), pp.40-48.

Siddiq, E., L. Vemireddy and J. Nagaraju. 2012. Basmati rices: genetics, breeding and trade. Agricultural Research 1: 25-36.

Smith, A.M., K. Denyer and C. Martin. 1997. The synthesis of the starch granule. Annual review of plant biology, 48(1), pp.67-87.

Srivastava, H.K. 2000. Nuclear Control and Mitochondrial Transcript Processing with Relevance to Cytoplasmic Male Sterility in Higher Plants. Current Science. 79(2): 176-186.

Su, Y., Y. Rao, S. Hu, Y. Yang, Z. Gao, G Zhang, et al. 2011. Map-based cloning proves qGC-6, a major QTL for gel consistency of japonica/indica cross, responds by Waxy in rice (*Oryza sativa* L.). Theoretical and Applied Genetics, 123(5), p.859.

Suwannaporn, P. and A. Linnemann. 2008. Rice-eating quality among consumers in different rice grain preference countries. Journal of Sensory Studies 23: 1-13.

Swamy, B.M., K. Kaladhar, N.S. Rani, G. Prasad, B. Viraktamath, G.A. Reddy and N. Sarla. 2012. QTL analysis for grain quality traits in 2 BC2F2 populations derived from crosses between *Oryza sativa* cv Swarna and 2 accessions of *O. nivara*. Journal of Heredity 103: 442-452.

Tabkhkar, N., B. Rabiei and A. Sabouri. 2012. Genetic diversity of rice cultivars by microsatellite markers tightly linked to cooking and eating quality. Australian Journal of Crop Science 6.

Tan, Y., J. Li, S. Yu, Y. Xing, C. Xu and Q. Zhang. 1999. The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theoretical and Applied Genetics. 99(3-4): 642-648.

Tang, W., T. Wu, J. Ye, J. Sun, Y. Jiang, J. Yu, J. Tang, G. Chen, et al. 2016. SNP-based analysis of genetic diversity reveals important alleles associated with seed size in rice. BMC Plant Biology. 16(1): 93.

Thakare, I., A. Mehta, J. Patel and S. Takle. 2010. Combining ability analysis for yield and grain quality traits in rice hybrids. Journal of Rice Research. 3: 1-5.

Tian, R., G.H. Jiang, L.H. Shen, L.Q. Wang and Y.Q. He. 2005. Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Molecular Breeding 15: 117-124.

Tripathi, A.K., A. Pareek, S.K. Sopory and S.L. Singla-Pareek. 2012. Narrowing down the targets for yield improvement in rice under normal and abiotic stress conditions via expression profiling of yield-related genes. Rice: *5*(1), p.37.

Tyagi, S., R. Mir, H. Kaur, P. Chhuneja, B. Ramesh, H. Balyan and P. Gupta. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). Molecular Breeding. 34(1): 167-175.

Umemoto, T., M. Yano, H. Satoh, A. Shomura and Y. Nakamura. 2002. Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theoretical and Applied Genetics.104: pp.1-8.

Umemoto, T., N. Aoki, H. Lin, Y. Nakamura, N. Inouchi, Y. Sato, et al. 2004. Natural variation in rice starch synthase IIa affects enzyme and starch properties. Functional Plant Biology. 31: pp.671-684.

Vaughan, D.A., P.L. Sanchez, J. Ushiki, A. Kaga and N. Tomooka. 2004. Asian rice and weedy evolutionary perspective. In J. Gressel, ed. Crop ferality and volunteerism. CRC Press, New York: p. 257-277.

Vithyashini, L. and H. Wickramasinghe. 2016. Genetic diversity of seed storage proteins of rice (*Oryza sativa* L.) varieties in Sri Lanka. Tropical Agricultural Research 27 (1): 49 – 58.

Von Mark, V.C., A. Kilian and D.A. Dierig. 2013. Development of DArT marker platforms and genetic diversity assessment of the US collection of the new oilseed crop *lesquerella* and related species. Plos One 8: e64062.

Wambugu, P., M.N. Ndjiondjop, A. Furtado and R. Henry. 2017. Sequencing of bulks of segregants allows dissection of genetic control of amylose content in rice. Plant Biotechnology Journal.pp.1-11

Wang, L., W. Liu, Y. Xu, Y. He, L. Luo, Y. Xing, C. Xu and Q. Zhang. 2007. Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain. Theoretical and Applied Genetics. 115(4): 463-476.

Wang, P., Y. Xing, Z. Li and S. Yu. 2012. Improving rice yield and quality by QTL pyramiding. Molecular Breeding 29: 903-913.

Ward, R.M., Q. Gao, H. de Bruyn, R.G. Gilbert and M.A. Fitzgerald. 2006. Improved methods for the structural analysis of the amylose-rich fraction from rice flour. Biomacromolecules 7: 866-876.

Wang, Z.Y., F.Q. Zheng, G.Z. Shen, J.P. Gao, D.P. Snustad, M.G. Li, et al.1995. The amylose content in rice endosperm is related to the post-transcriptional regulation of the Waxy gene. The Plant Journal, 7(4), pp.613-622.

Waters, D.L., R.J. Henry, R.F. Reinke and M.A. Fitzgerald. 2006. Gelatinization temperature of rice explained by polymorphisms in starch synthase. Plant Biotechnology Journal, 4(1), pp.115-122.

Weber, D., R. Rohilla and U. Singh. 2000. Chemistry and biochemistry of aroma in scented rice. Aromatic Rices: 29-46.

Wesley, I., B. Osborne, R. Anderssen, S. Delwiche and R. Graybosch. 2003. Chemometric localization approach to NIR measurement of apparent amylose content of ground wheat. Cereal Chemistry 80: 462-467.

Xie, X., F. Jin, M. Song, J. Suh, H. Hwang, Y. Kim, S. McCouch and S. Ahn. 2008. Fine mapping of a yield enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* x *O. rufipogon* cross. Theoretical and Applied Genetics. 116: 613-622.

Xing, Y. and Q. Zhang. 2010. Genetic and molecular bases of rice yield. Annual Review of Plant Biology. 61: 421-442.

Xu, F., G. Zhang, C. Tong, X. Sun, H. Corke, M. Sun, et al. 2013. Association mapping of starch physicochemical properties with starch biosynthesizing genes in waxy rice (*Oryza sativa* L.). Journal of Agricultural and Food Chemistry, 61(42), pp.10110-10117.

Xu, Y. and J.H. Crouch. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Science 48: 391-407.

Xu, Y., Z. Shen, Y. Chen and L. Zhu. 1995. Distorted segregations of RFLP markers and their distribution on chromosomes in an indica-japonica F2 population of rice (*Oryza sativa L*.). Acta Botanica Sinica 37: 91-96.

Yacouba, N.T., B. Yu, G. Gao, Q. Zhang, C. Xu, J. Xiao and Y. He. 2013. QTL analysis of eating quality and cooking process of rice using a new RIL population derived from a cross between Minghui 63 and Khao Dawk Mali105. Australian Journal of Crop Science 7: 2036.

Yamamoto, T., J. Yonemaru and M. Yano. 2009. Towards the understanding of complex traits in rice: substantially or superficially? DNA Research. 16(3): 141-154.

Yano, M. and T. Sasaki. 1997. Genetic and molecular dissection of quantitative traits in rice. (*Oryza sativa* L.): From Molecule to Plant. Springer. p. 145-153.

Yi, M., K.T. Nwe, A. Vanavichit, W. Chai-arree and T. Toojinda. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113: 178-186.

Zahid, M.A., M. Akhter, M. Sabar, Z. Manzoor and T. Awan. 2006. Correlation and path analysis studies of yield and economic traits in Basmati rice (*Oryza sativa* L.). Asian Journal of Plant Science. 5(4): 643-645.

Zhang, Z., M. Li, Y. Fang, F. Liu, Y. Lu, Q. Meng, et al. 2012. Diversification of the Waxy gene is closely related to variations in rice eating and cooking quality. Plant Molecular Biology Reporter.30 (2): pp.462-469.

Zheng, X., J.G. Wu, X.Y Lou, H.M. Xu and C.H. Shi. 2008. The QTL analysis on maternal and endosperm genome and their environmental interactions for characters of cooking quality in rice (*Oryza sativa* L.). Theoretical and Applied Genetics.116: 335–42.

Zhou, P., Y. Tan, Y. He, C. Xu and Q. Zhang. 2003. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theoretical and Applied Genetics. 106(2): 326-331.

2. Appraisal of major determinants of rice production and productivity and farmers’ choice of ideotypes in South Sudan: implications for breeding and policy interventions

Abstract

Rice (*Oryza sativa* L.) is an important staple crop in South Sudan. Current rice consumption in the country is approximately 23,000 tonnes/year of which more than 75% is imported. The study investigated farmers’ perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated rice agro-ecologies in South Sudan to guide breeding and policy interventions. Data were gathered through participatory rural approaches and a formal structured survey involving 136 rice farmers from major rice growing areas of South Sudan. Farmers generally cultivated old rice varieties with low yields varying from 0.4 to 1.6 t ha^{-1}. Pair-wise ranking based on respondents score indicated that yield, early maturity, cooking and eating quality, nutritional importance and drought tolerance as the most desirable traits of rice ideotypes. Further, imported rice varieties were best ranked for their sweet and appealing taste, rich aroma, grain shape and size, swelling capacity and non-stickiness during cooking. Major perceived constraints to rice production were unreliable rainfall, poor access to credit facilities, poor soil and water management practices, poor rice storage facilities, inadequate and poor processing machines as well as limited technical skills in rice production. The results show a need for integration of farmers’ and stakeholders’ preferences in variety development process and the desirability of releasing site-specific rice cultivars given the differences in bio-physical, socio-cultural and farmers’ preferences across major rice growing areas of South Sudan.

Keywords: *Oryza sativa* L., Participatory approaches, Farmer perceptions, Agro-morphological traits, Cooking and eating quality traits.

2.1 Introduction

Rice (*Oryza sativa* L.) is the staple food for over half of the world's population and is ranked as the world's number one human food crop (Anuonye et al., 2016). In South Sudan, rice is considered among the first four dominant staple cereals after sorghum, maize, and millet in terms of consumption. Results from the 2009 National Baseline Household Survey (NBS) suggested that more than 75% of rural households consume cereals. Furthermore, in the last two decades, South Sudan (formerly Sudan), was a net exporter of agricultural produce to regional markets and the potential of agriculture in stimulating economic growth was emphasized. However, due to civil war-related destruction, poor infrastructure and lack of investment in the agriculture sector, South Sudan is now a net importer of food. It currently imports as much as 50% of its food needs, including 40% of its cereals from neighbouring countries, particularly Uganda and Kenya. Total food imports are estimated to be in the range of US$ 200-300 million a year (AfDB, 2013). The national consumption of rice in South Sudan is approximated at 23,000 tonnes/year and the average per capita consumption is projected as 3 kg/year (AfDB, 2013).

It is also reported that South Sudan receives all its rice imports through Uganda (Nzomoi and Anderson, 2013), which acts as an important transit corridor for rice shipment of both locally produced and imported rice. Despite the importance of rice in South Sudan, local production is low. Further, introduced varieties do not meet the standards of imported rice in physical, cooking and eating qualities and therefore do not offer competitive prices in the market. A major challenge of the rice sector in the country is therefore how to produce sufficient and affordable high quality rice that not only meets the preferences of its fast-growing and increasingly urbanized population, but also competes favourably with imported rice. In addition, consumer preferences may be variable and closely associated with the historical and socio-cultural factors of a given region (Son et al., 2014), such that quality rice in one region may be considered to be of poor quality in yet another region. A study by Nzomoi and Anderson (2013) on rice markets in East Africa suggested that released rice varieties are not widely adopted because, in most cases, farmers and consumers may not prefer the taste or aroma of the introduced variety. For example in Uganda, consumers prefer aromatic to non-aromatic rice, sticky to non-sticky rice, unbroken to broken, bulging after cooking to rice that does not bulge, and white milled rice to brown. Thus, grain quality may be based on certain objective or subjective criteria that are of relative importance to the end-user. In general, the most important grain quality traits that are common to all end-users include appearance, milling quality, cooking, processing quality and nutritional quality (Lou et al., 2009). In light of this, knowledge on end-user tastes and preferences and identifying traits that farmers value in the selection of rice cultivars are important considerations for goal setting in a plant breeding programme (Virk et al., 2003).

Furthermore, the development of new high yielding varieties cannot have an appreciable impact unless the selection takes into account end-user qualities. Thus, researchers have become increasingly aware that incorporating end-user preferred qualities in technology development may substantially enhance chances of adoption of the technology (Joshi et al., 2002; Sié et al., 2012). Given this strategy, participatory plant breeding (PPB) approaches have been widely embraced in plant breeding programmes to engage farmers in variety selection process and have proven to be successful in eliciting farmers' preferred varieties and encouraging farmer variety uptake (Witcombe et al., 1996; Joshi et al., 2002; Asante et al., 2013). In addition, participatory rural appraisal (PRA) has been useful in identifying farmers' crop production constraints and variety preferences during the early phases of a crop improvement programme (Lamo, 2010; Mzengeza, 2010; Sibiya, 2010). This ensures development of rice cultivars tailored for specific adaptation (Joshi et al., 2002) which encourages competitiveness of locally produced rice and thus provides a mechanism to return more of the benefits associated with improved quality rice to the farmer. Thus the aim of this study was to investigate farmers' perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated ecologies in South Sudan to guide breeding and policy interventions.

2.2 Research methodology

2.2.1 Study area

In South Sudan, rice is cultivated under two production systems, namely; rainfed upland (in the Greenbelt agro-ecological zone) and irrigated lowland (in the western flood plains Agro-ecological zone), where the latter provides the greatest potential for rice cultivation. This study was conducted in five payams (which are administrative units comparable to townships) of South Sudan that cut across the two common rice ecologies. Under irrigated lowland rice ecology, Aweil Rice Scheme being the most predominant was selected, while in the rainfed upland rice ecology, Yambio County, comprising of Yambio center, Bangasu, Gangura and Lirangu payams were identified. The selected sites are representative of major rice growing areas of South Sudan.

The Aweil Rice Scheme in Aweil North County of Northern Bahr el Ghazal State is naturally covered with shrub and sparse trees with open deciduous woodland in the south, and receives a unimodal rainfall pattern with 800 to 1000 mm rainfall annually. The rice scheme lies within the flood plains agro-ecological zone and provides the greatest potential for lowland rice cultivation in South Sudan. It is located at $08^0$46'48'N latitude and $27^0$24'00 E longitude at an elevation of 425 m above sea level (asl) with an extensive irrigated lowland rice area of about 5,000 hectares. The soil is characteristic of black cotton soil, while the average monthly maximum temperatures vary slightly from 33.8ºC in March to 36.0ºC in September, and an average monthly minimum from 17.6ºC in September to 10.5ºC in December. The land along the river floods annually, though the floods are of variable volume and duration.

Yambio County is located along the tropical rain-forest belt within the Greenbelt agro-ecological zone of South Sudan at $04^{0}33'54'$ N latitude and $28^{0}22'30'$ E longitude with altitude of 650 m asl. The area receives a bimodal rainfall pattern with annual mean total rainfall of about 1443 mm and the soils are characteristic of ferrous soils with heavy deposits of fertile clay soils (Hoffmann et al., 2012).

2.2.2 Sampling procedure and participants

A simple random sampling technique was used to select rice farmers with at least 5 years of rice farming experience. Farmers were selected from across two common rice agro-ecologies of South Sudan, namely; the irrigated lowland rice cultivation in Aweil rice scheme and the rainfed rice production in Yambio County. Four payams were selected in Yambio County, namely; Gangura, Yambi center, Lirangu and Bangasu. The sample size comprised of 136 individual rice farmers (123 male and 13 female) selected from the two common rice growing sites (Table 2.1). Further, to provide a forum for joint identification of challenges and opportunities in the rice sector, focus group discussions (FGDs) were conducted (one at each payam) and brought together key stakeholders that included farmers, County Agriculture Commissioners, extension officers, traders and millers. The total number of participants in the group discussions was 60 (45 male and 15 female). In general, there were more male respondents than females in both the structured survey and group discussions (Table 2.1). The research team comprised of two scientists and ten extension officers (2 extension officers per site). The enumerators and facilitators were mainly local extension officers working in the study areas and were selected based on their familiarity with the local language, culture and farming activities at the respective sites.

Table 2.1: Total number of participants interviewed disaggregated by gender for structured survey and focus group discussions in selected rice growing areas

Payam	Male	Female	Total
Formal survey			
Aweil rice scheme	20	3	23
Gangura	28	4	32
Yambio center	21	3	24
Lirangu	39	1	40
Bangasu	15	2	17
Total	**123 (90.4%)**	**13 (9.6%)**	**136**
Focus group discussions			
Aweil rice scheme	7	2	9
Gangura	8	4	12
Yambio center	10	2	12
Lirangu	13	4	17
Bangasu	7	3	10
Total	**45 (75%)**	**15 (25%)**	**60**

2.2.3 Data collection

Primary data for the set objectives were collected through structured and pre-tested questionnaires (Appendix 5) and participatory learning approaches. Participatory rural appraisal tools were used in characterizing the bio-physical and socio-economic status of the rice farming households, including key informant interviews, focus group discussions and transect walks during farmer field observations to further validate data generated from individual interviews. Additional qualitative and quantitative data were gathered for preferred rice characteristics and cooking and eating qualities required by rice farmers and other stakeholders. Furthermore, data were collected on a number of variables, including demographic information and socio-economic indicators. Enumerators were guided through the questionnaires and focus group discussions points by the principal investigator to ensure clarity and to establish a common understanding of the exercise. The questionnaire was pre-tested on a small group of farmers and adjustments were made where necessary.

Group discussions allowed for joint identification and prioritization of challenges and opportunities aimed at enhancing local rice production. In addition, the discussions also focussed on outstanding issues emanating from individual farmer interviews. In one of the group discussions in Gangura payam, a rice cooking and eating quality exercise was conducted with a panel of 12 participants (8 male and 4 female) to capture stakeholders' general views on rice cooking and taste qualities and to provide for greater in depth discussion on the same. In this exercise, test materials included imported rice varieties ('Basmati', 'Pakistan', and 'China') and locally cultivated rice cultivars ('NERICA 4' and

‘NERICA 1’) which were considered as controls. The materials were acquired from the local market where imported rice was observed to be slightly more expensive than the locally produced rice.

The test materials were treated equally during the cooking process and were branded with letters to avoid any bias. Thereafter, stakeholders were able to score the cultivars following a linear scale of 1 (most preferred) to 5 (least preferred) for cooking and eating quality giving reasons for like or dislike. Furthermore, a participatory approach was used as a means to identify farmers’ needs in a cultivar and to expose stakeholders to new upland and lowland rice cultivars. The most important agro-morphological attributes were revealed and explained to the participants. From the traits chosen, participants gave weighting of the importance of each trait giving reasons for like or dislike. To assess how participants select traits that they consider in the selection of rice varieties to plant, score cards were used to distribute to each trait according to its importance following a linear scale of 1 (most preferred) to 5 (least preferred).

2.2.4 Data analysis

Descriptive statistics using frequency, means and percentages were calculated for different variables to explore relationships. Statistical analyses of both quantitative and qualitative data were performed in IBM SPSS Statistics version 21.0 (SPSS, 2012) computer package. Chi-square test for association was used to test for independence between rice growing sites and different variables. Data generated from focus group discussions was tallied, ranked and compared using matrix and pair-wise ranking procedures. Spearman’s rank correlation was used to test for consistency of ranks across sites.

2.3 Results

2.3.1 Demographics and socio-economic aspects

Significant differences ($P<0.001$) were observed for household relationship and number of individuals per household across the rice growing sites with implications on availability of farm labour, where most of the respondents (52%) were within the age range of 21-40 years (Table 2.2). In addition, the bulk of the respondents had attained primary education (58%) with a few others who had only achieved Ordinary School Certificate (15%) as the highest level of training (Table 2.2). Significant differences were also observed for land size allocated for rice production ($P<0.01$) and category of popular rice grown ($P<0.001$) across sites where a great number of rice farmers interviewed cultivated local landraces both in Aweil rice scheme (48%) and Yambio County (81%). Common rice varieties cultivated in Aweil rice scheme were ‘BG400-1’ and ‘BR4’, while in Yambio County, popular rice landraces were ‘Ruanya’ and ‘Zamburu’. The yields of the landraces were observed to range between 0.4-1.6 t ha^{-1} for both upland and lowland cultivars (Table 2.2).

Table 2.2: Farmer and household information for Aweil rice scheme and Yambio County in South Sudan

			Payams in Yambio County							
Variable	Class	Aweil rice scheme	Gangura	Yambio Center	Lirangu	Bangasu	Total counts	DF	Chi-square value	*P*-Value
Age (years)	<20	0	0	0	4	1	5	12	16.007	0.191
	21-40	17	15	13	17	18	70			
	41-60	6	14	11	16	7	54			
	61-80	0	3	0	3	1	7			
Household relationship	Head	11	31	24	40	14	120	12	53.988	<0.001
	Spouse	6	1	0	0	3	10			
	Child	3	0	0	0	0	3			
	Relative	3	0	0	0	0	3			
Household size (number of individuals)	<5	8	2	12	12	4	38	12	50.699	<0.001
	:6-10	15	18	6	16	2	57			
	:11-15	0	11	2	6	3	22			
	>15	0	1	4	6	8	19			
Level of education	None	9	9	5	5	6	34	12	16.048	0.189
	Primary (Grade 1-7)	9	19	16	28	7	79			
	Ordinary school certificate (Form 1-4)	3	4	3	6	4	20			
	Certificate (Agricultural training)	2	0	0	1	0	3			
Total farm size (hectares)	<1	12	17	14	20	11	74	8	9.183	0.327
	1.1-2.5	11	13	7	13	3	47			
	>2.5	0	2	3	7	3	15			
Land size allocated for rice (hectares)	0.01-0.1	0	2	0	0	3	5	12	29.786	<0.01
	0.1-1.0	15	29	23	34	16	112			
	1.0-2.0	8	3	0	5	1	17			
	>2.0	0	0	1	1	0	2			
Variety cultivated	Local landraces	11	25	21	32	14	103	8	39.254	<0.001
	Improved varieties	0	5	1	7	1	14			
	Local & improved	12	2	2	1	2	19			
Estimated yield (tonnes/ha)	0.4-1.0	9	10	10	14	4	47	12	15.842	0.199
	1.0-1.6	11	15	5	17	5	53			
	1.6-2.2	1	3	0	2	2	8			
	2.2-2.8	2	4	9	7	6	28			

2.3.2 Farmer staple crops and cropping systems

Results of chi-square test for independence revealed that crops grown by farmers did not differ significantly across the rice growing sites (Table 2.3). Within the lowland rice ecology, rice was grown entirely as a sole crop and was considered an important food and cash crop. Second to rice, sorghum was most preferred as a food crop followed by groundnut, maize and sesame (Table 2.3). Commonly cultivated rice and sorghum varieties were late maturing (>6 months). Sorghum was commonly intercropped with sesame, while maize was mainly cultivated in limited areas close to the homesteads and often consumed green. Groundnut which makes an important contribution to the household diet and also an important cash crop was mostly cultivated in sandy soils.

Upland rice in Yambio County (comprising Gangura, Yambio center, Lirangu and Bangasu payams) was widely grown as an intercrop with maize or cassava. Rice was generally considered an essential food crop and an important socio-cultural crop. Other important crops cultivated in Yambio County were groundnuts, maize, cassava and sorghum (Table 2.3). Similar to Aweil rice scheme, late maturing (<6 months) rice and sorghum varieties were predominant in Yambio County.

Table 2.3: Farmers' preferences (%) for staple crops across lowland and upland rice ecologies in South Sudan

					Sites	Mean across sites	Overall Rank ł
Crop	Aweil rice scheme	Gangura	Yambio center	Rirangu	Bangasu		
Sorghum	65.2	15.6	16.7	12.5	11.8	24.4	4
Cassava	0.0	37.5	45.8	15.0	11.8	22.0	5
Maize	17.4	53.1	79.2	55.0	17.6	44.5	3
Groundnut	39.1	46.9	83.3	47.5	23.5	48.1	2
Beans	0.0	6.3	12.5	7.5	0.0	5.3	8
Sesame	8.7	9.4	8.3	7.5	5.9	8.0	7
Millet	0.0	12.5	33.3	10.0	11.8	13.5	6
Rice	100.0	96.9	75.0	87.5	88.2	89.5	1
Overall mean						31.9	
DF						28.0	
Chi-square						56.0	
P-value						0.229^{ns}	

ł Overall rank based on percent mean values across sites. ł 'Overall rank 1 = best, to overall rank 8 = worst'. ns=Non-significant (*P*>0.05)

2.3.3 Farmers' desired rice variety attributes and their level of importance

At the lowland rice production site in Aweil rice scheme, farmers identified the four most important desirable variety characteristics as early maturity, phenotypic acceptability, yield and nutritional importance (Table 2.4). Whereas, in the upland rice growing sites within Yambio County, farmers in Gangura payam suggested yield, nutritional importance, pest resistance and early maturity as the most desirable cultivar traits. In Yambio centre payam, farmers opted for yield, cooking and eating quality, nutritional importance and drought tolerance as the most important traits of a variety. Conversely, in Lirangu payam, farmers advocated for yield, early maturity, cooking and eating quality and drought tolerance. Furthermore, in Bangasu payam, farmers identified improved cooking and eating quality, yield, early maturity and drought tolerance as the most preferred traits of a rice variety (Table 2.4). Ranking of mean scores across sites in order of importance revealed that yield, early maturity, cooking and eating quality, nutritional importance and drought tolerance were most desirable cultivar traits preferred by respondents (Table 2.5). Furthermore, spearman's rank correlation coefficient (r) suggested significant negative correlation (r=-0.64; *P*<0.01) in the consistency of ranking order of most desirable variety traits preferred by respondents across sites (Table 2.5).

Table 2.4: Pair-wise ranking of most desirable variety traits by respondents in lowland and upland rice ecologies in South Sudan

	Trait	A	B	C	D	E	F	G	H	Score ǂ	Rank
i)	***Aweil rice scheme***										
A	Nutritional importance	-	B	A	A	A	F	A	H	4	4
B	Early maturity		-	B	B	B	B	B	B	7	1
C	Drought tolerance			-	C	C	F	C	H	3	5
D	Pest/insect resistant				-	D	F	D	H	2	6
E	Disease resistant					-	F	G	H	0	8
F	Yield						-	F	H	5	3
G	Cooking and eating quality							-	H	1	7
H	Phenotypic acceptability								-	6	2
ii)	***Gangura***										
A	Nutritional importance	-	A	A	A	A	F	A	A	6	2
B	Early maturity		-	B	D	B	F	B	B	4	4
C	Drought tolerance			-	D	E	F	G	C	1	6
D	Pest/insect resistant				-	D	F	D	D	5	3
E	Disease resistant					-	F	G	H	1	6
F	Yield						-	F	F	7	1
G	Cooking and eating quality							-	G	3	5
H	Phenotypic acceptability								-	1	6
iii)	***Yambio center***										
A	Nutritional importance	-	A	A	A	A	F	G	A	5	3
B	Early maturity		-	C	D	E	F	G	B	1	7
C	Drought tolerance			-	C	C	F	G	C	4	4
D	Pest/insect resistant				-	D	F	G	D	3	5
E	Disease resistant					-	F	G	E	2	6
F	Yield						-	F	F	7	1
G	Cooking and eating quality							-	G	6	2
H	Phenotypic acceptability								-	0	8
iv)	***Lirangu***										
A	Nutritional importance	-	B	C	D	E	F	G	H	0	8
B	Early maturity		-	B	B	B	F	B	B	6	2
C	Drought tolerance			-	C	C	F	G	C	4	4
D	Pest/insect resistant				-	D	F	G	H	2	6
E	Disease resistant					-	F	G	H	1	7
F	Yield						-	F	F	7	1
G	Cooking and eating quality							-	G	5	3
H	Phenotypic acceptability								-	3	5
v)	***Bangasu***										
A	Nutritional importance	-	B	C	D	E	F	G	A	1	7
B	Early maturity		-	B	B	B	F	G	B	5	3
C	Drought tolerance			-	C	C	F	G	C	4	4
D	Pest/insect resistant				-	D	F	G	D	3	5
E	Disease resistant					-	F	G	E	2	6
F	Yield						-	G	F	6	2
G	Cooking and eating quality							-	G	7	1
H	Phenotypic acceptability								-	0	8

ł Letters correspond to traits listed along the column. ǂ The score is given by the frequency of the letter representing the trait. 'Rank 1 = most desirable, to rank 8 = least desirable'

Table 2.5: Pair-wise ranking of most desirable traits of varieties by respondents across lowland and upland rice ecologies in South Sudan

Trait	Score ł					Overall mean	
	Aweil rice scheme	Gangura	Yambio center	Lirangu	Bangasu	Score	Rank ǂ
Yield	5	7	7	7	6	6.4	1
Early maturity	7	4	1	6	5	4.6	2
Cooking and eating quality	1	3	6	5	7	4.4	3
Nutritional importance	4	6	5	0	1	3.2	4
Drought tolerance	3	1	4	4	4	3.2	4
Pest and insect resistant	2	5	3	2	3	3.0	5
Phenotypic acceptability	6	1	0	3	0	2.0	6
Disease resistance	0	1	2	1	2	1.2	7
Overall mean score						3.50	
DF						28.00	
Spearman’s rank correlation coefficient (r)						-0.64**	

ł Scores are generated from S2 Table. ǂ 'Rank 1 = most desirable, to rank 7 = least desirable'.
**Correlation is significant at the 0.01 level (2-tailed).

2.3.4 Stakeholder preferred rice cooking and eating quality traits

To understand stakeholder perceptions for cooking and eating quality traits, both imported and locally cultivated rice cultivars were subjected to cooking and eating quality tests. The best ranked rice varieties were imported varieties, namely; ‘Pakistan’, ‘Basmati’ and ‘China’ rice (Table 2.6). ‘Pakistan’ rice variety was preferred for its “sweet” taste, swelling capacity, aroma, grain shape and size and non-stickiness. On the other hand, ‘Basmati’ rice variety was desired for its grain shape and size, appealing sweet taste, non-stickiness and aroma, while ‘China’ rice variety was selected for its appealing golden colour, aroma, sweet taste, non-stickiness and swelling capacity. Among the two lowly ranked locally cultivated rice cultivars, ‘NERICA 1’ was preferred to ‘NERICA 4’ for its aroma, grain colour and less water use during cooking (Table 2.6). It was also noted that the price of imported rice in the market was slightly higher than the price of locally produced rice.

Table 2.6: Matrix ranking of stakeholder variety preferences for cooking and eating quality attributes during a focus group discussion

	Individual rank					Rank	Overall	Preference	
Variety	1	2	3	4	5	index	rank ɫ	Like	Dislike
A (Pakistan)	4	1	1	3	1	26	1	Sweet taste, swelling capacity, grain shape and size, aroma and non-sticky	None
B (Basmati)	1	5	0	1	3	30	2	Grain shape and size, sweet taste, appealing, non-sticky and aroma	None
C (China)	3	2	1	0	4	30	2	Aroma, taste, non-sticky, grain colour, swelling capacity,	Grain shape and size, require more water to cook
D (NERICA 1)	1	0	4	4	1	34	3	Grain colour, aroma, sweet taste, swelling capacity, and require less water to cook	Sticky, Grain shape and size
E (NERICA 4)	1	2	4	2	1	30	2	Sticky, swelling capacity, sweet taste	Non-aromatic, Sticky, require more water to cook, Grain shape and size

ɫ 'Overall rank 1 = best, to overall rank 3 = worst'. In parenthesis are the cultivar common names.

2.3.5 Desirable rice agro-morphological traits

Results of matrix ranking of agro-morphological traits among ten lowland and ten upland cultivars is presented in Tables 2.7 and 2.8. The best two selected lowland cultivars were ‘NERICA-L-6’ and ‘K-85’. ‘NERICA-L-6’ was most preferred for early maturity, large panicles and enhanced tillering capacity. The least preferred lowland rice cultivar was ‘1189’ because it had small panicles and was late maturing (Table 2.7).

The best ranked upland rice cultivars were ‘NERICA 1’ and ‘ART3-8L6P3-2-3-B’, which were selected for early maturity and enhanced tillering capacity. The least preferred upland rice cultivar (‘ART3-7L9P8-3-5-B-B-2’) was disliked for having small panicles and few productive tillers (Table 2.8).

The most desirable agro-morphological attributes in both lowland and upland rice cultivars were early maturity, large panicles and enhanced tillering capacity. Overall, most locally grown rice cultivars across all rice growing sites were characterised as late maturing (>6 months) with low yields (0.4-1.6 tonnes/hectare) and less potential for providing surplus produce for the market. Rice is an important food and cash crop in South Sudan. However, rice production is unable to meet the growing local demand yet the locally produced rice faces stiff competition from imported rice.

Table 2.7: Matrix ranking of stakeholder preferences for agro-morphological traits in lowland rice cultivars during a focus group discussion

	Individual rank			Rank index	Overall rank ƚ	Preferences	
Variety	1	2	3			Like	Dislike
A (326104)	0	4	0	8	5	Early maturity,	Short, small panicles
B (NERICA-L-19)	0	1	1	5	3	Large panicles and early maturity	Tall, Few tillers
C (Supa 1052)	0	1	1	5	3	Large grains, strong stem, large panicle	Tall, late maturing
D (K-85)	0	1	0	2	2	Grain shape, grain size, early maturity	Small panicles,
E (1189)	4	2	2	14	7	Grain shape	Small panicle, late maturing, less tillers
F (Kumboka)	2	0	1	5	3	Early maturity and large panicles, aromatic	Less tillers
G (Supa-TZ)	0	0	2	6	4	Late maturity, aromatic, large panicles	Tall, few tillers
H (NERICA-L-6)	1	0	0	1	1	Early maturity, large panicles, tillering capacity	Grain shape, grain size
I (TXD-306)	2	0	1	5	3	Early maturity, aromatic	Tall, Weak stems
J (Wita 9)	1	1	2	9	6	Short, grain shape, grain size	Late maturing, susceptible to blast, few tillers

ƚ 'Overall rank 1 = best, to overall rank 7 = worst'. In parenthesis are the cultivar lineages or common names.

Table 2.8: Matrix ranking of stakeholder preferences for agro-morphological traits in upland rice cultivars during a focus group discussion

Variety	Individual rank			Rank index	Overall rank ł	Preference	
	1	2	3			Like	Dislike
A (P5 H6)	0	4	0	8	4	Grain colour,	Few tillers
B (ART2-4L3P1-2-1)	0	1	1	5	2	Early maturity, large panicles	Weak stems, few tillers,
C (ART3 -8L6P3-2-3-B)	0	1	0	2	1	Tillering capacity, early maturity,	Grain shape, grain size
D (NERICA 1)	0	1	0	2	1	Early maturity, aromatic, tillering capacity	Grain shape, grain size
E (ART3-7L9P8-3-5-B-B-2)	4	2	2	14	6	Early maturity	Small panicle size, few tillers,
F (ART3 -7L3P3-B-B-2)	2	0	1	5	2	Tillering capacity, large grain size	Grain shape
G (SCRIDO 06-2-4-3-4-5)	0	0	2	6	3	Tillering capacity, large panicles	Late maturing
H (ART25-3-29-2-B)	1	0	1	5	2	Large grains, medium height	Grain shape, grain size, non-aromatic
I (NERICA 4)	2	0	1	5	2	Early maturity, tillering capacity, large panicles	Grain shape, grain size, non-aromatic
J (ART12-L2P2-20-3-1-1)	1	1	2	9	5	Grain shape, grain size	Few tillers, late maturing, small panicles

ł 'Overall rank 1 = best, to overall rank 6 = worst'. In parenthesis are the cultivar lineages or common names.

2.3.6 Farmers' perceived constraints to rice production across lowland and upland rice ecologies

Significant differences (P<0.001) were observed in the consistency of ranking order of major rice production constraints across lowland and upland rice growing sites (Table 2.9). The most important constraints across lowland and upland rice ecologies were; unreliable rainfall, poor access to credit facilities, poor soil and water management practices, poor rice storage facilities, inadequate and poor processing machines as well as limited technical skills in rice production (Table 2.9). Poor soil and water management practices was identified as a major constraint to lowland rice production, while unreliable rainfall was mentioned as a critical factor affecting upland rice production (Table 2.9).

Table 2.9: Ranking of major rice production constraints across lowland and upland rice production sites in South Sudan

	ł Mean scores						
Constraint	Aweil	Gangura	Yambio center	Rirangu	Bangasu	Overall Mean	ǂ Rank
Unreliable rainfall	1.96	1.91	1.96	1.98	1.94	1.95	1
Poor access to credit facilities	1.87	1.88	1.88	1.90	1.88	1.88	2
Poor soil and water management practices	1.96	1.88	1.83	1.85	1.82	1.87	3
Birds damage	1.91	1.81	1.75	1.90	1.94	1.86	4
Inadequate technical skills	1.78	1.75	1.88	1.93	1.88	1.84	5
Inadequate and poor processing machines	1.87	1.78	1.88	1.83	1.76	1.82	6
Poor storage facilities	1.87	1.78	1.71	1.75	1.88	1.80	7
Pests and disease infestation	1.74	1.78	1.75	1.65	1.65	1.71	8
High costs of production	1.70	1.63	1.75	1.65	1.65	1.68	9
Low yielding varieties	1.96	1.47	1.38	1.73	1.71	1.65	10
Poor roads and transport facilities	1.74	1.47	1.58	1.73	1.65	1.63	11
Lack of lucrative markets	1.87	1.34	1.38	1.30	1.53	1.48	12
Limited access to farm inputs	1.39	1.31	1.33	1.55	1.47	1.41	13
Overall mean						1.32	
DF						48.00	
Spearman's rank correlation coefficient (r)						-0.83**	

ǂ Rank based on overall mean scores across sites. 'Rank 1 = most important, to rank 13 = least important'. **Correlation is significant at the 0.01 level (2-tailed).

2.4 Discussion

Women play a prominent role in farming in South Sudan, providing close to 80% of farm labour (AfDB, 2013). However, in this study more males participated than females. This is strongly attributed to the traditional set-up and cultures where men often as house-hold heads take lead in farm planning and decision making and are custodians of common household wealth as observed in this study. Significant differences were observed for number of individuals per household. The majority of sampled farmers consisted of a youthful age group with the potential for increased agricultural production and productivity. Although some respondents indicated having attended short courses in agricultural training commonly offered by agricultural extension officers and non-governmental organizations, specialized formal training in rice production and management was inadequate. Farming was based on small, hand cultivated units of less than one hectare per household. Despite the huge potential for rice cultivation in South Sudan, farmers use traditional and unimproved farming practices and unimproved seeds limiting potential production and productivity of the crop. In both rice ecologies, majority of the rice farmers cultivate using obsolete rice varieties or landraces which have been recycled over the years. Although landraces are an important element of plant genetic resources (Gyawali et al., 2010), they are generally characterized by low yields. In spite of efforts to introduce new varieties by development partners and NGOs, most of the introduced varieties did not go through testing for adaptability. Also, these varieties were not evaluated for their agronomic performance and attributes and hence are rarely adopted by rice farmers. Therefore, this study suggests options for enhancing improved rice variety uptake by incorporating farmer and consumer desirable traits in the desired cultivar through participatory breeding approaches.

Next to rice, sorghum and groundnut were the most preferred crops at the lowland rice growing site, while maize and groundnut were desirable at the upland rice growing sites. The differences may be explained by the suitability of the soils for production of the desired crop and the economic and socio-cultural importance of the crop. Furthermore, rice was also mainly cultivated as a sole crop in the lowland rice ecology and as an intercrop in the upland rice ecology, where in the former; rice was considered an important food and cash crop, whereas in the latter, rice was valued as an essential food and socio-cultural crop. Across all sites, farmers mainly cultivated local landraces or old crop varieties probably due to inadequate exposure to new and modern cultivars or the absence of acceptable alternatives to their landraces (Witcombe et al., 1996). Although, there have been efforts to introduce improved rice varieties mainly by non-governmental organizations, the failure to produce varieties adapted to varied production conditions or with traits valued by farmers and consumers may explain the limited variety adoption (Dalton and Timothy, 2004; Witcombe et al., 2004). In both rice growing systems, farmers commonly used their own seed saved from the previous harvest and rarely used fertilizers, pesticides or herbicides.

Variability in farmer variety preferences across sites may be influenced by historical and socio-cultural factors (Son et al., 2014) as depicted in the present study. Consequently, this may provide important breeding considerations in generating site-specific rice cultivars in South Sudan. Yield and early maturity which were selected as the most desirable attributes across the rice growing sites relate to preferable farmer cultivar traits that offer a clear yield advantage with potential for double cropping. However, this does not always translate into increased revenue, thus consumer preference is often considered a major driver of widespread uptake of a new variety (Calingacion et al., 2015) and is directly associated with grain quality traits such as cooking and eating quality in rice (Oko and Dambaba, 2012). Hence, it is important to note that rice producers' and consumers' alike are important drivers of rice production and choice of variety. Consequently, variety attributes are important considerations in designing breeding strategies for developing rice varieties that incorporate both farmers' and consumers' preferences for enhanced variety uptake.

Overall, imported rice were highly preferred mainly for the "sweet" and appealing taste, grain shape and size, aroma, swelling capacity and non-stickiness during cooking, while the locally produced rice cultivars were less preferred based on their non-appealing grain shape and size, taste and stickiness during cooking. Similar results were reported by Asante et al. (2013) in West Africa and Kikuchi et al. (2016) in East Africa. Gender differences were also observed where different rice varieties appealed differently to either men or women. The men generally preferred varieties that are long and slender because it is preferred in the market while the women selected for varieties that are aromatic and swell when cooked.

In this study, the local cultivars which are recently introduced, farmer preferred and adapted to the upland rice ecology of South Sudan do not match the imported rice in end-user qualities. Furthermore, imported rice was generally observed to be slightly more expensive than the locally produced rice. Locally produced rice does not offer competitive prices in the market being inferior in processing, cooking and eating qualities. A study by Demont and Ndour (2015) on upgrading of rice value chains as evidenced from 11 African markets concluded that generally urban rice consumers were willing to pay more for rice with superior intrinsic and extrinsic quality attributes. Furthermore, Demont (2013) suggests that in order to make domestic rice competitive to imported rice, African governments will need to invest more resources in rice value-addition. Consequently, this provides a great opportunity for varietal quality improvement by African rice producing countries to reduce rice imports and generate higher export revenues. Additionally, consumers' choice of rice varieties is largely determined by the grain cooking and eating qualities (Oko and Dambaba, 2012). Although preferences may vary from one group of consumers to another, rice grains with a pleasant fragrance and a soft texture often attracts higher market prices (Yi et al., 2009).

Rainfall is erratic across rice growing areas in South Sudan. Aweil rice scheme which lies within the flood plain agro-ecological zone of South Sudan frequently experiences torrential rains that results in flooding for most part of the rice growing season. Erratic and torrential rains are a major cause of crop failure and therefore viewed as a major challenge in rice production across all rice ecologies in the country. Further, absence or inadequate access to credit facilities was suggested as an important constraint in rice production. Agriculture is generally viewed as a risky business by most financing institutions in South Sudan, while there are very few of these institutions in the country that support agricultural investment, most are located in major towns that are not easily accessible by rice growers. Furthermore, poor soil and water management practices were identified as major constraints to rice production. As noted in this study, farmers rarely used fertilizers although most of the soils are depleted in nutrients, contributing to the observed low rice yields. Additionally, at the irrigated lowland site at Aweil rice scheme, irrigation and water management practices were unsatisfactory hence a major limiting factor in rice production. Another major setback mentioned by rice growers was grain damage by birds in both lowland and upland rice growing areas. Birds' damage was more pronounced in rice fields that were sown late, predisposing the crop to damage by migrating birds. Also, the lack of capacity building in various aspects of rice cultivation was identified as a major constraint to rice production in South Sudan. Farmers generally lacked basic skills in improved rice production and management practices and commonly followed traditional methods of rice production such as broadcasting as opposed to improved planting methods, for example, dibbling and line planting methods that can offer improved yields.

2.5 Conclusion

The present study revealed important considerations for the rice breeding programme in South Sudan and provided options for policy interventions essential for boosting the rice industry in the country. Majority of the rice farmers were relatively young, within the economically active age group but were less exposed to specialised formal training in rice production and management. The study also highlighted the fact that rice farming was largely dominated by traditional rice farming methods including broadcasting and use of farmer saved seed or recycling of old rice varieties despite efforts to introduce new rice varieties. The low uptake of new rice varieties largely emanates from lack of incorporating farmer and consumer preferences in these varieties, hence farmers are rarely exposed to rice varieties that provide acceptable alternatives to their landraces. The criteria for selecting rice varieties were influenced by locations and gender differences. For example, the men preferred rice varieties that are long and slender and fetch high market prices, while the women selected for varieties that are aromatic and swell when cooked. The study suggests improved farmers' and stakeholders' participation in the variety development process, incorporating their desired grain quality traits and preferences in the variety of choice. African governments are encouraged to invest more resources in rice value-addition in order to make domestic rice competitive to imported rice and enable African rice farmers to access urban markets. Furthermore, policy options that encourage increased access to farm inputs through agro-dealer networks and judicious use of fertilizers will help to enhance rice production per unit hectare. The study also suggests generating site-specific rice cultivars for South Sudan given the differences in bio-physical, socio-cultural and farmer preferred traits across rice production sites. The study further recognizes the important role of research in generating appropriate rice technologies while advocating for policy measures that encourage quality rice seed production and distribution in the country.

References

AfDB. 2013. African Development Bank Group.South Sudan: An Infrastructure Action Plan - A Program for Sustained Strong Economic Growth. pp.131-167.

Anuonye, J., O. Daramola, C. Chinma and O. Banso. 2016. Effects of processing methods on physicochemical, functional and sensory properties of Ofada rice. International Journal of Biotechnology and Food Science. 4(1): 7-14.

Asante, M.D., B.O. Asante, G.K. Acheampong, S.K. Offei, V. Gracen, H. Adu-Dapaah and E.Y. Danquah. 2013. Farmer and consumer preferences for rice in the Ashanti region of Ghana: Implications for rice breeding in West Africa. Journal of Plant Breeding and Crop Science 5: 229-238.

Calingacion, M., L. Fang, L. Quiatchon-Baeza, R. Mumm, A. Riedel, R.D. Hall and M. Fitzgerald. 2015. Delving deeper into technological innovations to understand differences in rice quality. Rice 8: 6.

Dalton and Timothy. 2004. A household hedonic model of rice traits: economic values from farmers in West Africa. Agricultural Economics 31: 149-159.

Demont, M. 2013. Reversing urban bias in African rice markets: A review of 19 National Rice Development Strategies. Global Food Security. 2(3): 172-181.

Demont, M. and M. Ndour. 2015. Upgrading rice value chains: Experimental evidence from 11 African markets. Global Food Security. 5: 70-76.

Gyawali, S., B. Sthapit, B. Bhandari, J. Bajracharya, P. Shrestha, M. Upadhyay and D. Jarvis. 2010. Participatory crop improvement and formal release of Jethobudho rice landrace in Nepal. Euphytica 176: 59-78.

Hoffmann, I., L. Blum, L. Kern, E. Mewes and R. Oelmann. 2012. Achieving Food Security in a Post Conflict ContextAlbrecht Daniel Thaer-Institut für Agrar-und Gartenbauwissenschaften.

Joshi, K.D., B. Sthapit, M. Subedi and J.R. Witcombe. 2002. Participatory plant breeding in 10 rice in Nepal. Farmers, Scientists and Plant Breeding: Integrating Knowledge and Practice: 239-267.

Kikuchi, M., Y. Haneishi, A. Maruyama, K. Tokida, G. Asea and T. Tsuboi. 2016. The competitiveness of domestic rice production in East Africa: A domestic resource cost approach in Uganda. Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS) 117: 57-72.

Lamo, J. 2010. Genetic studies on drought tolerance and grain shattering in rice. University of KwaZulu-Natal.

Lou, J., L. Chen, G. Yue, Q. Lou, H. Mei, L. Xiong and L. Luo. 2009. QTL mapping of grain quality traits in rice. Journal of Cereal Science 50: 145-151.

Mzengeza, T. 2010. Genetic studies of grain and morphological traits in early generation crosses of Malawi rice (*Oryza sativa* L.) Landraces and NERICA varieties. University of KwaZulu-Natal.

Nzomoi, J. and I. Anderson. 2013. The Rice Market in East Africa. Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists, September 22-25, 2013,Hammamet,Tunisia. P.14.

Oko, A. and N. Dambaba. 2012. Rice cooking quality and physico-chemical characteristics: a comparative analysis of selected local and newly introduced rice varieties in Ebonyi State, Nigeria. Food and Public Health 2: 43-49.

Sibiya, J. 2010. Breeding investigations for resistance to Phaeosphaeria leaf spot (PLS) and other important foliar diseases and a study of yield stability in African maize germplasm (Doctoral Dissertation).

Sié, M., K. Sanni, K. Futakuchi, B. Manneh, S. Mande, R. Vodouhé, S. Dogbe, K. Drame, et al. 2012. Towards a rational use of African rice (*Oryza glaberrima* Steud.) for breeding in Sub-Saharan Africa. Genes, Genomes and Genomics. 6(1): 1-7.

Son, J.S., V.B. Do, K.O. Kim, M.S. Cho, T. Suwonsichon and D. Valentin. 2014. Understanding the effect of culture on food representations using word associations: the case of “rice” and “good rice”. Food Quality and Preference 31: 38-48.

SPSS, IBM. 2012. Statistics for windows, version 20.0. IBM Corp., Armonk, NY.

Virk, D., D. Singh, S. Prasad, J. Gangwar and J. Witcombe. 2003. Collaborative and consultative participatory plant breeding of rice for the rainfed uplands of eastern India. Euphytica 132: 95-108.

Witcombe, J., K. Joshi, S. Gyawali, K. Devkota and A. Subedi. 2004. Impacts of participatory crop improvement in the low-altitude regions of Nepal.

Witcombe, J.R., A. Joshi, K.D. Joshi and B. Sthapit. 1996. Farmer participatory crop improvement. I. Varietal selection and breeding methods and their impact on biodiversity. Experimental agriculture 32: 445-460.

Yi, M., K.T. Nwe, A. Vanavichit, W. Chai-arree and T. Toojinda. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113: 178-186.

3. Genetic analysis of elite upland rice genotypes using DArTseq markers and comparative analysis of agro-morphological and grain quality traits

Abstract

A study was conducted to assess the genetic diversity and relationship present among 36 elite upland rice genotypes using Diversity Arrays Technology Sequencing (DArTseq) markers and agro-morphological and grain quality traits. Genetic diversity estimates amongst test genotypes based on DArTseq-derived SNP markers revealed only two distinct clusters suggesting genetic relatedness. This was associated with a low mean fixation index (F_{st}) of 0.188. Analysis of variance based on agro-morphological and grain quality traits indicated highly significant differences ($P< 0.001$) among the tested genotypes. Principal component and cluster analyses using the two trait groupings indicated that major contributors to grain yield per plant were the number of productive tillers per plant, number of panicles per plant, grain width, number of grains per panicle, panicle length, grain length to width ratio, percent spikelet fertility and weight of 1000 grains. Grain yield had significant positive correlation with number of panicles per plant, percent spikelet fertility and number of grains per panicle. Path coefficient analysis showed that direct selection for number of grains per panicle can be effective in enhancing grain yield. Grain length expressed the greatest maximum direct positive effect on amylose content, while grain width revealed the largest negative effect on this trait. Number of grains per panicle, number of panicles per plant, weight of 1000 grains, percent spikelet fertility and grain length can be considered as important selection criteria for genetic improvement of grain yield and cooking quality in rice. Overall, using DArTseq-derived SNP markers and agro-morphological and grain quality traits the following genetically diverse rice genotypes: ‘P5H6’, ‘NAMCHE 6’ and ‘ART3-7L9P8-3-5-B-B-2’ were selected for direct production or use in breeding.

Keywords: Upland rice, DArTseq markers, Grain quality, Genetic diversity, Path coefficient analysis.

3.1 Introduction

Rice (*Oryza sativa* L.) is the staple food for over half of the world's population (Anuonye et al., 2016) and the fourth most important and widely cultivated cereal crop in sub-Saharan Africa (SSA) (Masette et al., 2015). In South Sudan, rice is the most consumed staple crop after sorghum and maize. Despite the importance of rice in South Sudan, local production is low. Grain quality of introduced varieties does not meet the standards of imported rice in physical, cooking and eating qualities and therefore the varieties do not offer competitive prices in the market. According to African Development Bank (AfDB (2013), South Sudan imports some 50% of its food needs including 40% of its cereals from neighbouring countries including Kenya and Uganda. Furthermore, the country receives all its rice imports through Uganda which provides an important passage for shipment of both Ugandan locally produced and imported rice (Nzomoi and Anderson, 2013). A major challenge in the rice sector in South Sudan is how to produce sufficient and affordable high quality rice that not only meets the preferences of its fast-growing and increasingly urbanized population, but also that competes favourably with imported rice.

Increased rice production, productivity and ultimate market demand largely depends on producers' and consumers' tastes and preferences in a rice cultivar (Hossain et al., 2015). Grain quality traits common to all users include grain appearance, milling quality, cooking and processing quality, and nutritional quality. Cooking and eating qualities are most critical in the market for consumer acceptance. The two most important quality indicators for these traits are amylose content (AC) and gelatinization temperature (GT), measured indirectly as alkali spreading value (ASV) (Roy et al., 2012).

There is a growing demand for better quality rice with improved eating and cooking qualities important in determining its economic value in the market (Phing et al., 2016). Consequently, Concepcion et al. (2015) suggests that farmer adoption and consumer acceptance of a released variety is largely controlled by grain quality, while Premkumar et al. (2016) underscore the importance of not only yield but grain quality as a major determinant for the success of a rice variety. Traditional rice varieties with good grain quality but low yield potential are still widely grown in many rice producing countries despite the availability of improved varieties with relatively high yield potential but lower grain quality (Fitzgerald et al., 2009). Hence, potential new varieties must meet or exceed standards of locally preferred cultivars in both yield and quality traits before being considered for release (Concepcion et al., 2015). Improving grain yield and quality are important considerations in a rice breeding programme (Borba et al., 2010). However this presents a major challenge given the quantitative nature of these traits and the influence of genotype-by-environment interactions (Yi et al., 2009; Wang et al., 2012).

Molecular marker technology provides options for improving selection strategies (Akhtar et al., 2010), and facilitates the selection of traits during the breeding process because they are reliable and unaffected by environmental conditions (Yi et al., 2009). Also, for a successful marker-assisted breeding programme, a detailed phenotypic and genotypic understanding of parental genotypes is necessary (Balakrishnan et al., 2016). Recent advancements in next generation sequencing (NGS) technologies has enabled the use of genotyping-by-sequencing (GBS) as a promising genomic approach for simultaneous exploration of plant genetic diversity and molecular marker discovery (Elshire et al., 2011; Poland and Rife, 2012; He et al., 2014). Furthermore, microarray-based markers such as Diversity Arrays Technology (DArT) markers are preferred for construction of high-density maps, quantitative trait loci (QTL) mapping and genetic diversity analysis because of their efficiency and low cost (Gupta et al., 2008). To further enhance options for sequencing of complex genes, the DArTseq marker platform was developed by combining the complexity reduction of the DArT marker technology with high-throughput NGS technologies (Sánchez-Sevilla et al., 2015).

The magnitude of genetic variability present and the extent to which the desirable characters are heritable largely determines the success of any plant breeding programme (Vanaja and Luckins, 2006). To initiate a marker-assisted rice breeding programme, selection of genetically divergent parents and a clear understanding of the genetics of yield and grain quality related factors are critical in making the best use of the relationships in selection. Additionally, correlation studies is considered a useful tool for direct or indirect selection of interrelated characters (Abdala et al., 2016; Dhurai et al., 2016; Premkumar et al., 2016) in a breeding programme. Data generated from correlation analysis can be augmented by path analysis, which further splits the genotypic correlation coefficient into the measure of direct and indirect effects (Premkumar et al., 2016). This is particularly important in the early phases of a breeding programme in screening of new introductions to identify superior lines for further improvement (Saha et al., 2016). Hence, knowledge on the associations between yield and grain quality attributing factors is critical to breeders for enhancing selection and tailoring rice hybridization programmes. Therefore, the present study was undertaken to assess the genetic diversity and relationship present among 36 elite upland rice genotypes using DArTseq markers and to perform a comparative analysis for agro-morphological and grain quality traits.

3.2 Materials and methods

3.2.1 Plant materials

The study used a total of 36 elite upland rice lines (Table 3.1). Genotypes were acquired from AfricaRice (ARC), National Crops Resources Research Institute (NaCRRI-Uganda) and Institut d'Economie Rurale (IER-Mali). A popular landrace variety (‘Mbume’) and four recently introduced farmer preferred upland rice cultivars (‘NERICA 1’, ‘NERICA 4’, ‘NERICA 10’ and ‘DKAP27’) widely grown in South Sudan were included as comparative controls.

Table 3.1: Upland rice lines used in the study

Entry No.	Name/pedigree	Origin and abbreviation	Entry No.	Name/pedigree	Origin and abbreviation
1	SCRIDO 37-4-2-2-5	Madagascar (MDG)	19	ART3 -7L3P3-B-B-2	ARC
2	NAMCHE 2	NaCRRI (UG)	20	P23 H1	NaCRRI (UG)
3	P24 H10	NaCRRI (UG)	21	NAMCHE 4	NaCRRI (UG)
4	CT11891-3-3-3-M-1-2-2-M	CIAT	22	DKAP27	IER-Mali
5	P5 H6	NaCRRI (UG)	23	Mbume	Landrace (LDR)
6	NAMCHE 6	NaCRRI (UG)	24	NAMCHE 3	NaCRRI (UG)
7	ART10-1L15P1-4-3-1	ARC	25	ART25-3-29-2-B	ARC
8	ART2-4L3P1-2-1	ARC	26	WAC116xNERICA 4	IER-Mali
9	ART3-8L6P3-2-3-B	ARC	27	NAMCHE 1	NaCRRI (UG)
10	SCRIDO 06-2-4-3-4-5	Madagascar (MDG)	28	P29 H1	NaCRRI (UG)
11	ART3-8L6P3-2-2-B	NaCRRI (UG)	29	NAMCHE 5	NaCRRI (UG)
12	P27 H4	NaCRRI (UG)	30	ART12 -L4P7-21-4-B-3	ARC
13	P26 H1	NaCRRI (UG)	31	ART12-L2P2-20-3-1-1	ARC
14	NERICA 1	ARC	32	P24 H1	ARC
15	ART3-7L9P8-3-5-B-B-2	ARC	33	P62 H17	NaCRRI (UG)
16	P5 H14	NaCRRI (UG)	34	ART16-4-11-13-4	NaCRRI (UG)
17	P27 H3	NaCRRI (UG)	35	PCT-4\0\0\0>19-M-1-1-5-1-M	ARC
18	NERICA 10	ARC	36	NERICA 4	ARC

ł ARC, AfricaRice; CIAT, International Centre for Tropical Agriculture; NaCRRI, National Crops Resources Research Institute-Uganda; IER, Institut d'Economie Rurale-Mali.

3.2.2 Description of study site

An experiment was established at Yei Agricultural Research Station (YARS) which is located within the Greenbelt agro-ecological zone at 4^0 05″N latitude and 30^{0}41″E longitude at an altitude of 856 m. Annual mean total rainfall is about 1362 mm and a ferrous soil type is predominant.

3.2.3 Experimental design

The 36 elite upland rice lines were evaluated using a randomized complete block design (RCBD) with 3 replications. The experiments were conducted during the second rainy season (July-August, 2015). Planting was by direct-seeding with 3-5 seeds per hill, and later thinned to one seedling per hill 14-20 days after emergence (DAE). Each line was established in 3-row plots with 12 plants per row in a plot size of 1 m x 3 m and planting density of 20 cm between plants and 20 cm between rows. Standard cultural management practices including hand planting and hand weeding were uniformly applied throughout the crop growth period. The crops were fertilized with 25 kg N ha^{-1} at 20-25 DAE and the same rate at 40-45 DAE to enhance plant vigour.

3.2.4 Data collection

3.2.4.1 Quality traits

Genotypes were assessed for determinants of kernel quality including kernel size-shape, amylose content, alkali spreading value and other important agronomic characters on yield performance. Kernel quality was determined using dehusked grains. Kernels were classified on the basis of length (L) and width (W) for L/W ratio (shape) in three replicates using a vernier calliper following classification described by (Cruz and Khush, 2000). Amylose and amylopectin content of the starch was determined by the method of Gibson et al. (1997). Gelatinization temperature (GT) was assessed indirectly as the alkali spreading value of hulled kernels as per modified procedure of Little et al. (1958).

3.2.4.2 Agro-morphological traits

Agronomic traits measured included days to heading (DH), plant height (PH), number of productive tillers per plant (NETP), number of grains per panicle (NGP), panicle length (PL), percent spikelet fertility (PSF), weight of 1000 grains (TGW) and grain yield (GY). Days to heading were recorded when 50% of the plants in each plot had flowered, while PH, NETP and NGP were measured at maturity and based on ten individual plants randomly selected in each plot. PH was measured from the soil surface to the tip of the panicle, while PL was measured from the node of the panicle to the tip of the panicle. Phenotypic acceptability and proneness to lodging was recorded visually according to the rice Standard Evaluation System (SES) described by IRRI (2002). Only the inner row was considered for measurement of GY in each plot, whilst the grain moisture content was adjusted to 14% and the GY per plot extrapolated to tonnes per hectare.

3.2.4.3 Quantification of amylose and amylopectin

Amylose and amylopectin content of the starch was determined by the method of Gibson et al. (1997) using a Megazyme amylose/amylopectin assay kit (K-AMYL 04/06, Megazyme International Ireland Ltd., Co. Wicklow, Ireland), which is a modification of a Con A method developed by Yun and Matheson (1990). The method is also modified from Morrison and Laignelet (1983) and uses an ethanol pre-treatment step to remove lipids prior to analysis. Initially, rice samples were dehusked and polished prior to milling. Twenty whole-milled rice kernels from each of the 36 rice genotypes were ground separately and accurately weighed (20-25 mg to the nearest 0.1 mg) into a 10 ml screw capped Kimax sample tube. One millilitre of dimethyl sulfoxide (DMSO) was added while gently stirring at low speed on a vortex mixer. Samples were heated in a boiling water bath for 15 minutes with intermittent high-speed stirring on a vortex mixer and allowed to cool for 5 minutes at room temperature. Two millilitres of 95% ethanol were added with continuous stirring on a vortex mixer. A further 4 millilitres of ethanol were added and allowed to mix and kept overnight or allowed to stand for 15 minutes. After precipitate formation, the tubes were centrifuged for 5 minutes at 2000 revolutions per minute (rpm), and supernatant discarded. Two millilitres DMSO was then added to the pellet with vortexing and heating in boiling water bath for another 15 minutes. Four millilitres of Con A solvent was immediately added and solution adjusted to 25 ml in volumetric flask by repeated washing with Con A solvent (this was labelled solution A). One millilitre of solution A was then pipetted into a 2 ml eppendorf microfuge tube with the addition of 0.5 ml Con A solution and allowed to stand at room temperature for one hour. The eppendorf tubes were then centrifuged for 10 minutes at 14000 rpm at room temperature. One millilitre of supernatant was transferred to a 15 ml centrifuge tube and 3 ml of sodium acetate buffer of pH 4.5 added. The tubes were heated in a boiling water bath for 5 minutes and allowed to equilibrate in a 40°C water bath for 5 minutes. About 0.1 ml of amyloglucosidase/α-amylase enzyme mixture was added and incubated at 40°C for 30 minutes. The tubes were then centrifuged at 2000 rpm for 5 minutes. To 1.0 ml aliquots of the supernatant, 4 ml of GOPOD reagent was added and incubated at 40^0C for 20 minutes. The absorbance of each sample and the D-glucose controls were read at 510 nm against the reagent blank. Total starch absorbance was determined by mixing 0.5 ml aliquots of solution A with 4 ml of sodium acetate buffer. A 0.1 ml of amyloglucosidase/ α -amylose solution was added and incubated for 10 minutes at 40^0C. One millilitre aliquots of this solution was transferred to glass test tubes, to which 4 ml GOPOD reagent was added and incubated for 20 minutes at 40^0C. The incubation was performed concurrently with the samples and standards. Absorbance of samples was then read at 510 nm. Amylose content was then determined as follows;

Amylose, % (w/w)

$$= \frac{\text{Absorbance (Con A Supernatant)} x \ 6.15 \ x \ 100}{\text{Absorbance (Total Starch Aliquot)} 9.2 \text{ x } 1}$$

$$= \frac{\text{Absorbance (Con A Supernatant)} x \ 66.8}{\text{Absorbance (Total Starch Aliquot)}}$$

Where, 6.15 and 9.2 are dilution factors for the Con A and Total Starch extracts, respectively. The samples were then classified following standard procedures by Juliano (1971) with slight modifications, where; 3-9% amylose content indicates waxy to very low AC, 10-19% amylose content indicates low AC; 20-25% amylose content indicates intermediate AC, 26-30% amylose content indicates high-AC, while >31% amylose content indicates very high-AC.

3.2.4.4 Gelatinization temperature

Gelatinization temperature (GT) was assessed indirectly as the alkali spreading value of hulled kernels as per modified procedure of Little et al. (1958). Twelve whole grains, were immersed in petri-plates containing 1.7% KOH in such a way that no two grains were in contact with each other. The plates were then incubated for 24 h at room temperature. The ASV were determined by visual scoring of the appearance of the grains and disintegration on a 1–7 linear scale as described by Govindaraj et al. (2009), where; 1 = grains not affected, 2 = grains swollen, 3 = grains swollen, collar incomplete and narrow, 4 = grain swollen, collar complete and wide, 5 = grains split or segmented, collar complete and wide, 6 = grain dispersed, merging with collar and 7 = grain completely dispersed and intermingled. Grains swollen to the extent of a cottony centre and a cloudy collar were given an ASV score 4 and used as a check for scoring the rest of the samples. Since ASV is inversely related to GT the higher value of ASV was taken for low GT and vice versa. A rating of 1.00–2.99 was taken as high GT (>74°C), 3.00–4.99 as intermediate (69–74°C) and 5.00–7.00 as low GT (55–68°C) as referred in Govindaraj et al. (2009).

3.2.4.5 DNA isolation and genotyping

Total genomic DNA was isolated from three-week old leaves using the ZYMO research *Quick*-DNA™ Plant/Seed 96 Kit, where a single individual plant was considered for each accession. Subsequently, 40 µl of a 50 ng/µl DNA of each sample was sent to Diversity Arrays Technology (DArT) Pty Ltd, Australia ('http://www.diversityarrays.com/dart-map-sequences') for whole genome scan using DArTseq markers. Whole-genome genotyping was carried out using GBS technology as described by Elshire et al. (2011). A total of 18,927 DArTseq-derived SNP markers were used to genotype the 36 rice lines. The markers were integrated into a linkage map by inferring marker order and position from the consensus DArTseq map.

3.2.4.6 Data filtering and SNP calling

DArTseq-derived SNP markers were filtered to remove bad SNPs and genotypes using PLINK 1.9 software in MS window and R statistical software, where genotypes with > 30% missing data, SNP loci with >20% missing data (Figure 3.1) and rare SNPs with <5% minor allele frequencies (MAF) were pruned. Only 453 SNPs and 34 genotypes were considered after filtering and data quality control process.

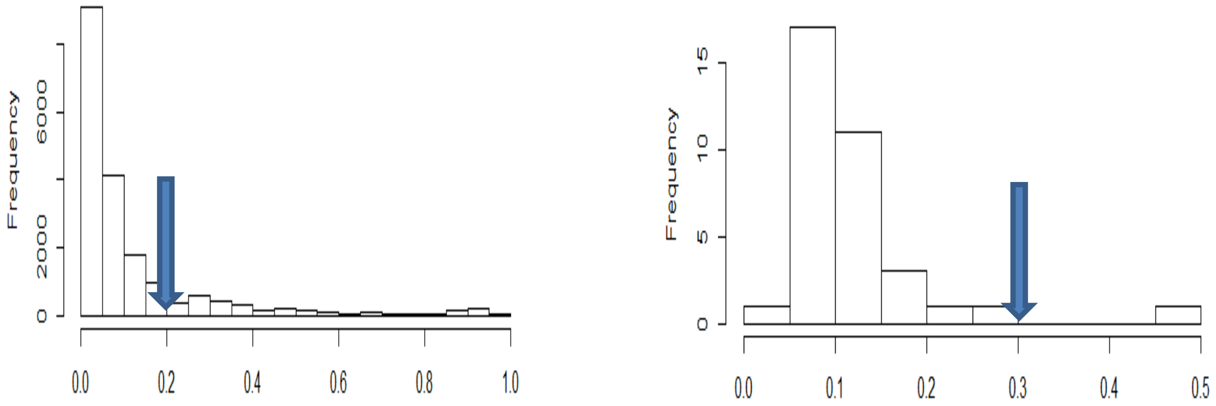


Figure 3.1: Frequency of SNPs (loci) with missing data (left) and frequency of genotypes with missing data (right)

3.2.5 Data analysis

3.2.5.1 Agronomic traits

Data analyses were performed using GenStat 14th Edition software (VSN, 2011) using the analysis of variance (ANOVA) procedure followed by mean comparisons for agronomic characters and grain quality traits. The linear model used was as follows;

$$Y_{ij} = \mu + T_i + \beta_j + \varepsilon_{ij}$$

Where Y_{ij} is any observation for which $_i$ is the treatment factor and $_j$ is the blocking factor, μ is the mean, T_i is the effect of treatment $_i$, β_j represents the effect of Block $_j$, ε_{ij} are the residuals. Mean separation was conducted using the least significance difference (LSD) at 5% probability. Ward's hierarchical clustering was used to assess the phenotypic diversity in rice lines based on their agro-morphological and grain quality attributes. Cluster analysis was performed using SPSS statistical software (IBM, 2012). The Z values were calculated from mean values and used for cluster analysis. Principal component analysis was used to further complement cluster analysis results. Pearson's correlation coefficients (r) among agro-morphological and grain quality traits were calculated using SPSS software following the method described by (Singh and Chaudhary, 1977). Data generated from correlation coefficient was augmented by path analysis to further split the genotypic correlation coefficient into direct and indirect effects as described by (Dewey and Lu, 1959). Information gathered was used to investigate relationships between grain yield and other important agro-morphological and grain quality traits.

3.2.5.2 Genetic analysis

The genetic structure and relationship among upland rice genotypes was investigated using 453 DArTseq-derived SNP markers distributed across the rice genome as described by Pritchard et al.

(2000). Bayesian clustering method was applied to identify clusters of genetically similar individuals using the software STRUCTURE version 2.3 (Pritchard et al., 2003) and further visualized with Structure Plot V2.0 (Ramasamy et al., 2014). The cluster values (K) were chosen ranging from 1 to 10 and ten independent runs for each value in order to obtain consistent results. The best K-value for estimating a suitable population size for the dataset was determined as K=2 based on the Evanno et al. (2005) method from STRUCTURE run. In addition, population differentiation due to genetic structure was assessed using a neighbour-joining (NJ) tree method (Saitou and Nei, 1987) generated by R statistical software. Analysis of molecular variance (AMOVA) was performed using GenAlEx V6.5 software (Peakall and Smouse, 2006). SNP data were numerically coded as follows: A= 1, C= 2, T= 3, G= 4 and missing data was coded as 0 as suggested in GenAlEx V6.5 user manual.

3.3 Results

3.3.1 Performance of tested rice genotypes

Significant differences were observed among the tested rice genotypes ($P<0.05$) for all agro-morphological traits except for number of panicles per plant (Table 3.2 and 3.3). Days to heading varied from 82 days (‘NERICA 10’) to 94 days (‘P62 H17’) (Table 3.3). Grain yield was highest in ‘ART10-1L15P1-4-3-1’ (5.1 t ha^{-1}) and lowest in ‘WAC x NERICA 4’ (1.6 t ha^{-1}).The mean number of panicles per plant ranged from 4.49 (‘WAC x NERICA 4’) to 9.99 (‘NAMCHE 6’), plant height ranged from 63.14 cm (‘ART3-8L6P3-2-3-B’) to 101.33 cm (‘CT11891-3-3-3-M-1-2-2-M’), number of effective tillers per plant from 6 (‘NAMCHE 6’) to 26 (‘NAMCHE 1’), panicle length from 16.34 cm (‘ART3-8L6P3-2-2-B’) to 22.0 cm (‘NERICA 10’), percent spikelet fertility from 0.75 (‘SCRIDO 06-2-4-3-4-5’) to 0.91 (‘P29 H1’), number of grains per panicle from 65.8 (‘ART12-L2P2-20-3-1-1’) to 132.4 (‘P27 H4’) and weight of 1000 grains from 23.77g (‘ART3-8L6P3-2-3-B’) to 34.25g (‘NERICA 10’) (Table 3.3).

Highly significant differences ($P<0.001$) were recorded for all grain quality traits of rice genotypes tested in the present study (Table 3.2 and 3.4). Grain width ranged from 1.05 mm (‘PCT-4\0\0\0>19-M-1-1-5-1-M’) to 1.91 mm (‘NAMCHE 6’), while grain length varied from 5.15 mm (‘ART3-8L6P3-2-3-B’) to 7.7 mm (‘ART2-4L3P1-2-1’) and grain length to width ratio from 2.89 (‘ART3-8L6P3-2-3-B’) to 6.16 (‘ART16-4-11-13-4’). Alkali spreading value (ASV) which is inversely related to gelatinization temperature (GT) ranged from 1.67 (‘P23 H1’) to 5.0 (‘SCRIDO 37-4-2-2-5’), which gives a GT range from high to low, respectively. Amylose content (AC) was highest in ‘ART12-L2P2-20-3-1-1’ (39.73%) and lowest in ‘P23 H1’ (15.8%), ranging from very high to low AC, respectively (Table 3.4).

Table 3.2: Mean squares and significant tests from analysis of variance of agro-morphological and grain quality traits among 36 rice genotypes

		Agro-morphological traits								
Source of variation	df	DH	PPP	PH	NETP	PL	PSF	NGP	TGW	GY
Genotype	35	49.4***	14.1^{ns}	603.0***	204.5**	17.4***	0.01*	2925.6***	53.9*	6.5***
Replication	2	17.5	5.2	1246.6	4536.0	8.6	0.01	508.2	70.1	0.8
Error		11.8	10.9	270.0	108.6	8.2	0.01	966.3	34.6	2.7

		Grain quality traits				
Source of variation	df	AC%	GL	GW	L/W	ASV
Genotype	35	105.4***	1.11***	0.2***	2.2***	1.9***
Replication	2	25.7	0.06	0.0	0.02	6.3
Error		13.9	0.00	0.0	0.01	0.2

[ǂ] DH, days to heading; PPP, panicles per plant; PH, plant height; NETP, number of effective tillers per plant; PL, panicle length; PSF, percent spikelet fertility; NGP, number of grains per panicle; TGW, weight of 1000 grains (g); GY, grain yield (t ha^{-1}); AC; amylose content; GL, grain length; GW, grain width; L/W, grain length to width ratio; ASV, alkali spreading value ; *,**,***,^{ns}, significant at $P<0.05$, $P<0.01$, $P<0.001$ and non-significant at $P>0.05$, respectively.

Table 3.3: Mean values for agro-morphological traits of 36 upland rice genotypes

Entry No.	Name/pedigree	DH	PPP	PH	NETP	PL	PSF	NGP	TGW	GY
1	SCRIDO 37-4-2-2-5	91.89	5.40	99.76	7.67	16.57	0.84	78.30	33.71	1.97
2	NAMCHE 2	90.33	5.65	78.96	14.33	17.41	0.81	74.80	26.24	1.67
3	P24 H10	90.33	6.98	86.42	15.89	19.56	0.83	109.40	29.71	3.79
4	CT11891-3-3-3-M-1-2-2-M	90.00	7.94	101.33	13.78	19.93	0.85	80.40	32.07	2.82
5	P5 H6	91.44	8.66	78.03	15.56	18.67	0.85	99.20	32.72	4.19
6	NAMCHE 6	88.67	9.99	82.18	6.00	19.11	0.90	86.20	31.04	4.32
7	ART10-1L15P1-4-3-1	92.33	9.08	72.22	16.11	20.22	0.86	129.80	27.24	5.10
8	ART2-4L3P1-2-1	93.22	5.92	86.62	10.56	19.92	0.82	85.90	29.89	2.27
9	ART3-8L6P3-2-3-B	92.33	9.71	63.14	17.00	19.27	0.85	93.70	23.77	2.90
10	SCRIDO 06-2-4-3-4-5	90.11	7.28	80.81	15.67	18.94	0.75	79.80	25.52	2.06
11	ART3-8L6P3-2-2-B	91.11	6.09	77.37	7.44	16.34	0.86	71.70	30.66	1.90
12	P27 H4	91.44	7.52	90.21	20.44	21.96	0.82	132.40	26.65	4.01
13	P26 H1	91.22	7.44	89.26	12.56	18.89	0.88	112.00	29.97	3.92
14	NERICA 1	90.78	8.06	84.13	13.67	20.28	0.78	89.20	30.62	2.73
15	ART3-7L9P8-3-5-B-B-2	91.67	7.52	87.53	22.00	18.67	0.90	130.70	30.88	5.05
16	P5 H14	86.78	9.02	94.66	20.56	19.70	0.84	97.30	25.54	2.90
17	P27 H3	91.44	6.83	84.48	16.89	19.53	0.85	106.80	27.32	3.07
18	NERICA 10	82.78	7.22	91.18	9.33	22.00	0.79	91.20	34.25	3.25
19	ART3 -7L3P3-B-B-2	90.44	6.27	87.22	14.67	19.36	0.81	93.90	28.38	2.17
20	P23 H1	92.22	7.56	90.69	13.11	17.21	0.87	80.30	31.06	2.70
21	NAMCHE 4	90.00	4.86	93.11	16.56	19.21	0.84	127.70	27.72	2.39
22	DKAP27	90.22	8.31	84.91	14.11	16.72	0.87	72.90	31.22	2.77
23	Mbume	90.00	7.11	88.40	24.56	18.39	0.89	85.30	27.07	2.68
24	NAMCHE 3	91.00	5.93	95.44	14.11	19.39	0.81	118.90	30.98	2.95
25	ART25-3-29-2-B	91.89	7.78	95.61	18.11	20.56	0.86	90.20	31.15	3.15
26	WACxNERICA 4	93.33	4.49	84.61	12.22	17.44	0.84	91.80	27.34	1.60
27	NAMCHE 1	93.67	7.82	93.00	26.00	17.33	0.87	74.20	32.35	2.89
28	P29 H1	84.22	7.75	92.56	24.56	19.48	0.91	86.60	28.63	3.11
29	NAMCHE 5	89.00	6.08	99.40	16.56	19.59	0.87	114.40	26.07	2.64
30	ART12 -L4P7-21-4-B-3	89.67	8.14	90.07	14.44	17.22	0.88	88.00	30.48	3.27
31	ART12-L2P2-20-3-1-1	93.33	7.14	95.84	11.89	16.82	0.85	65.80	28.88	1.97
32	P24 H1	87.89	6.40	96.54	18.44	17.84	0.89	89.40	30.04	2.74
33	P62 H17	93.89	6.48	84.24	9.89	18.62	0.91	89.20	29.75	2.67
34	ART16-4-11-13-4	89.56	6.62	76.62	16.33	19.53	0.87	116.40	30.21	3.62
35	PCT-4\0\0\0>19-M-1-1-5-1-M	90.33	6.74	79.32	20.67	17.58	0.91	97.90	30.22	3.53
36	NERICA 4	91.11	7.28	89.40	19.67	19.07	0.86	108.20	27.53	3.15
	Mean	90.55	7.20	87.37	15.59	18.84	0.85	95.60	29.36	3.00
	CV%	3.80	46.00	18.80	66.80	15.20	10.40	32.50	20.00	54.90
	S.e.d	1.62	1.56	7.75	4.91	1.35	0.04	14.65	2.78	0.78
	LSD (5%)	3.19	3.07	15.25	9.67	2.65	0.08	28.84	5.46	1.53
	F-value	4.18***	1.28^{ns}	2.23***	1.88**	2.13***	1.58*	3.03***	1.55*	2.41***

[†] DH, days to heading; PPP, panicles plant; PH, plant height; NETP, number of effective tillers per plant; PL, panicle length; PSF, percent spikelet fertility; NGP, number of grains/panicle; TGW, weight of 1000 grains (g); GY, grain yield (t ha^{-1}); *,***,^{ns}, denote significant differences at P<0.05, P<0.001 and non-significant, respectively.

Table 3.4: Mean values for grain quality traits of 36 upland rice genotypes

Entry No.	Name/Pedigree	AC%	GL(mm)	GW(mm)	L/W ratio	ASV
1	SCRIDO 37-4-2-2-5	24.34	5.70	1.15	4.97	5.00
2	NAMCHE 2	22.28	6.55	1.79	3.66	4.67
3	P24 H10	27.86	6.45	1.15	5.61	4.67
4	CT11891-3-3-3-M-1-2-2-M	30.90	7.65	1.50	5.10	4.00
5	P5 H6	22.71	5.75	1.41	4.08	3.67
6	NAMCHE 6	20.18	6.50	1.91	3.41	3.67
7	ART10-1L15P1-4-3-1	17.40	7.30	1.90	3.84	3.67
8	ART2-4L3P1-2-1	20.34	7.70	1.41	5.48	4.67
9	ART3-8L6P3-2-3-B	21.45	5.15	1.79	2.89	3.33
10	SCRIDO 06-2-4-3-4-5	16.77	6.20	1.79	3.46	3.33
11	ART3-8L6P3-2-2-B	25.68	6.60	1.15	5.75	3.67
12	P27 H4	37.67	6.40	1.58	4.06	2.67
13	P26 H1	24.55	6.85	1.89	3.63	4.33
14	NERICA 1	17.43	5.80	1.79	3.24	4.33
15	ART3-7L9P8-3-5-B-B-2	23.56	6.75	1.89	3.57	3.67
16	P5 H14	26.37	6.05	1.41	4.31	3.33
17	P27 H3	17.08	6.75	1.49	4.53	3.67
18	NERICA 10	26.91	6.95	1.22	5.70	2.67
19	ART3 -7L3P3-B-B-2	16.39	6.75	1.69	4.01	4.33
20	P23 H1	15.80	6.80	1.79	3.81	1.67
21	NAMCHE 4	18.46	6.75	1.41	4.79	3.67
22	DKAP27	24.35	5.65	1.78	3.17	3.33
23	Mbume	33.83	6.75	1.81	3.73	2.33
24	NAMCHE 3	20.92	5.95	1.55	3.84	4.33
25	ART25-3-29-2-B	28.95	6.40	1.81	3.55	3.67
26	WACxNERICA 4	27.83	6.65	1.41	4.72	2.33
27	NAMCHE 1	24.37	5.65	1.79	3.16	3.33
28	P29 H1	19.32	7.30	1.79	4.09	4.00
29	NAMCHE 5	26.76	5.75	1.49	3.86	4.00
30	ART12 -L4P7-21-4-B-3	17.02	6.50	1.79	3.63	3.33
31	ART12-L2P2-20-3-1-1	39.73	7.10	1.81	3.93	3.33
32	P24 H1	23.68	6.35	1.51	4.21	4.33
33	P62 H17	16.10	5.85	1.79	3.28	4.67
34	ART16-4-11-13-4	25.39	6.78	1.10	6.16	2.67
35	PCT-4\0\0\0>19-M-1-1-5-1-M	17.26	5.58	1.05	5.32	4.33
36	NERICA 4	26.91	5.85	1.59	3.69	2.33
	Mean	23.52	6.43	1.59	4.17	3.64
	CV%	15.90	0.80	1.40	2.40	10.80
	S.e.d	3.05	0.04	0.02	0.08	0.32
	LSD (0.05%)	6.08	0.08	0.04	0.16	0.64
	F-value	7.56***	476.43***	423.59***	223.80***	12.11***

ǂ AC; amylose content; GL, grain length; GW, grain width; L/W, grain length to width ratio; ASV, alkali spreading value ; ***, significant at P<0.001.

3.3.2 Relationships of upland rice genotypes as revealed by quantitative agro-morphological and grain quality traits

Principal components analysis bi-plots for PC1 versus PC2 accounted for 60.5% of the variation among the rice genotypes (Figure 3.2). Furthermore, principal component analysis yielded seven principal components that accounted for 83.6% of total variation among the test materials (Table 3.5). Furthermore, a breakdown of the cumulative variance value revealed contributions of 20.5%, 16.7%, 11.2%, 11.0%, 9.1%, 7.8% and 7.3% for PCA1, PCA2, PCA3, PCA4, PCA5, PCA6 and PCA7, respectively. The first, second, fourth and fifth PCA were correlated with grain yield and its contributing traits such as number of effective tillers per plant, number of panicles per plant, grain width, number of grains per panicle, panicle length, grain length to width ratio, percent spikelet fertility and weight of 1000 grains. The third and sixth PCA were correlated with AC and ASV, respectively, and plant height as their contributing trait. The seventh PCA was correlated with grain length and number of days to heading as its contributing trait.

Results obtained from PCA were further corroborated by cluster analysis using Unweighted Paired Group Method using Centroids (UPGMC) (Figure 3.3). Genotypes were grouped into seven distinct clusters that were well resolved in terms of agro-morphological characters and grain quality traits. Cluster 1 (3 genotypes) indicated above average weight of 1000 grains and intermediate to high AC and ASV values. Cluster 2 (11 genotypes) generally grouped together high yielding genotypes. Cluster 3 (seven genotypes) mainly consisted of tall and high tillering genotypes. Cluster 4 (12 genotypes) was dominated by below average yielding genotypes. Clusters 5, 6 and 7 emerged as the most distinct clusters, where Cluster 5 genotypes had the highest plant height (101.33 cm), Cluster 6 genotype recorded below average scores for days to heading, number of panicles per plant, number of effective tillers per plant, percent spikelet fertility, number of grains per panicle, weight of 1000 grains, grain yield, amylose content and grain length to width ratio, while Cluster 7 genotype had the lowest average yield (1.6 t ha^{-1}).

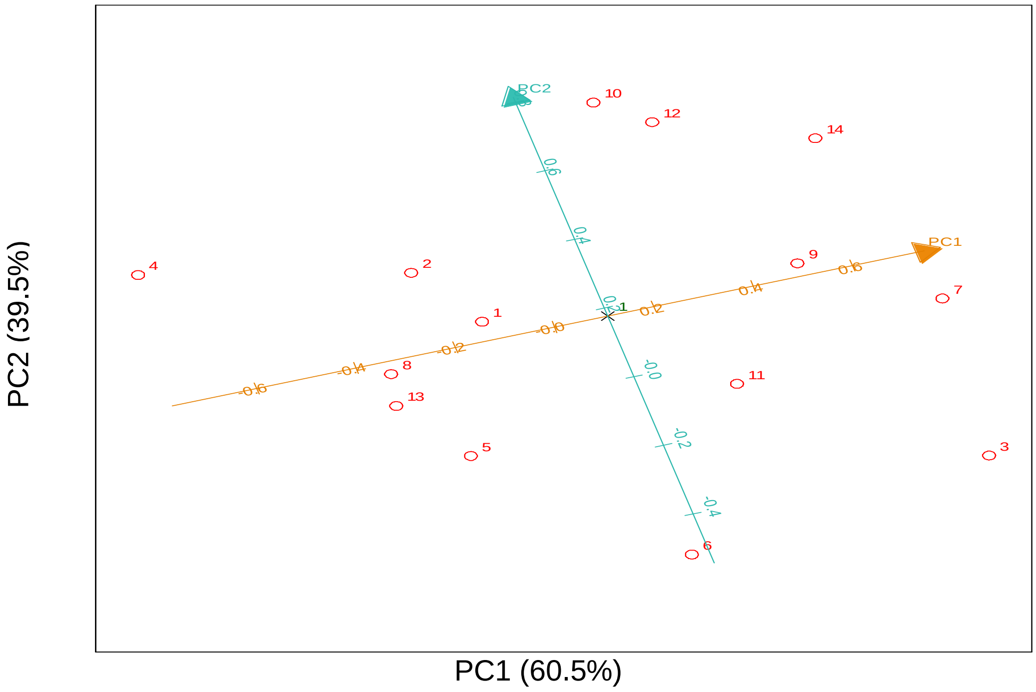


Figure 3.2 Principal components analysis bi-plot showing PC1 and PC2 accounting for 60.5% of the variation

Table 3.5: Loadings of PCA for grain yield and its components, and grain quality traits

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
AC	-0.130	0.231	0.695	-0.172	0.113	-0.165	0.111
GL	-0.222	0.401	0.226	0.060	-0.348	-0.051	0.630
GW	0.701	-0.407	0.215	-0.020	-0.384	0.199	0.278
L/W	-0.729	0.546	-0.125	0.086	0.190	-0.264	0.043
ASV	-0.258	-0.122	-0.467	0.242	-0.086	0.634	-0.031
DH	0.072	-0.508	-0.149	-0.125	0.345	0.033	0.594
PPP	0.740	0.039	0.015	0.300	-0.325	-0.317	-0.181
PH	-0.340	0.141	0.642	0.143	-0.122	0.559	-0.072
NETP	0.500	0.213	0.367	-0.198	0.413	0.223	-0.266
PL	0.251	0.756	-0.131	-0.251	-0.369	0.137	-0.017
PSF	0.292	-0.076	0.244	0.616	0.505	0.011	0.097
NGP	0.344	0.671	-0.280	-0.160	0.343	0.269	0.200
TGW	-0.356	0.053	0.053	0.783	-0.150	-0.003	-0.017
GY	0.633	0.538	-0.174	0.444	0.103	-0.035	0.130
Eigenvalues	2.868	2.335	1.571	1.542	1.276	1.097	1.019
Variance (%)	20.488	16.681	11.221	11.016	9.112	7.835	7.275
Cumulative variance (%)	20.488	37.169	48.389	59.406	68.517	76.352	83.627

[†]AC; amylose content; GL, grain length; GW, grain width; L/W, grain length to width ratio; ASV, alkali spreading value DH, days to heading; PPP, panicles per plant; PH, plant height; NETP, number of effective tillers per plant; PL, panicle length; PSF, percent spikelet fertility; NGP, number of grains per panicle; TGW, weight of 1000 grains; GY, grain yield.

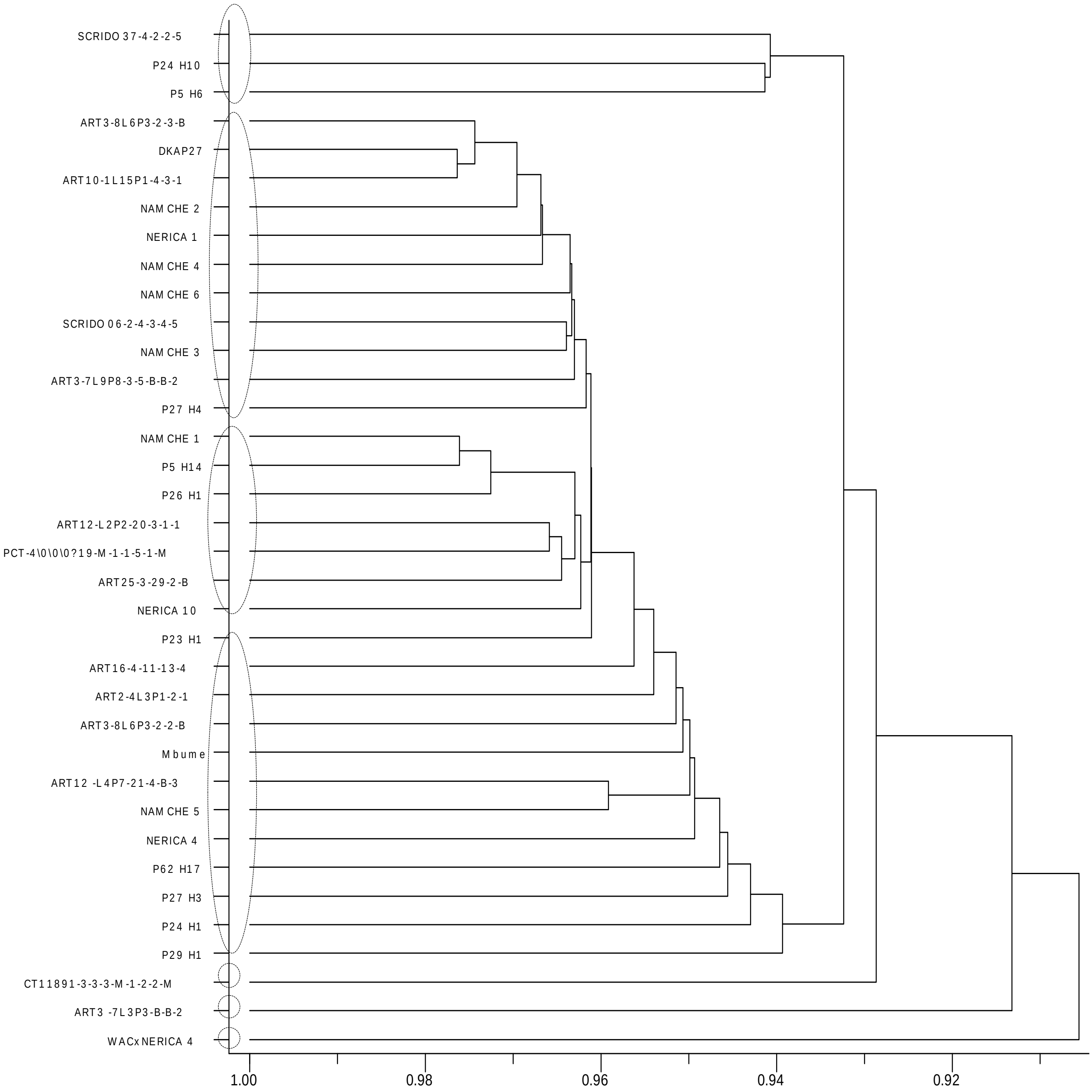


Figure 3.3: Unweighted Paired Group Method using Centroids (UPGMC) dendrogram showing the relationships of upland rice genotypes revealed by quantitative agro-morphological characters and grain quality traits

3.3.3 Correlations between grain yield with yield components and grain quality traits

Pearson’s correlation coefficients were calculated among nine agro-morphological characters and five grain quality traits (Table 3.6). Grain yield had significant positive correlation with number of panicles per plant, percent spikelet fertility and number of grains per panicle. Amylose content recorded significant positive correlation with plant height, while percent spikelet fertility had significant negative correlation with panicle length. Grain length to width ratio showed significant positive correlation with grain length and significant negative correlation with grain width and number of panicles per plant.

Path coefficient analysis using grain yield and amylose content as response variables and other agro-morphological and grain quality traits as causal variables is illustrated in Table 3.7 and 3.8.

Number of grains per panicle expressed the greatest maximum direct positive effect (0.715) on grain yield followed by number of panicles per plant (0.547), weight of 1000 grains (0.391), percent spikelet fertility (0.135) and number of effective tillers per plant (0.066). Plant height (-0.146) exhibited the largest negative direct effect on grain yield followed by panicle length (-0.068) and number of days to heading (-0.021). Number of panicles per plant indicated a positive indirect effect on grain yield through panicle length (0.102) and percent spikelet fertility (0.101), and a negative indirect effect through plant height (-0.159). Plant height recorded a positive indirect effect on grain yield through weight of 1000 grains (0.125) and a negative indirect effect through number of panicles per plant (-0.159). Number of effective tillers per plant expressed a positive indirect effect on grain yield through number of grains per panicle (0.206) and a negative indirect effect through weight of 1000 grains (-0.129). Panicle length indicated a positive indirect effect on grain yield through number of panicles per plant (0.102) and number of grains per panicle (0.103). Percent spikelet fertility had a positive indirect effect on grain yield through number of panicles per plant (0.101). Number of grains per panicle indicated a negative indirect effect on grain yield through weight of 1000 grains (-0.170) and a positive indirect effect through number of effective tillers per plant (0.206) and panicle length (0.103). Weight of 1000 grains exhibited a positive indirect effect on grain yield through plant height (0.125) and a negative indirect effect through number of grains per panicle (-0.170) and number of effective tillers per plant (-0.129).

Grain length exhibited the greatest maximum direct positive effect on amylose content (0.471), while grain width revealed the largest negative effect on amylose content (-0.862) followed by grain length to width ratio (-0.773) and alkali spreading value (-0.306). Grain length had a negative indirect effect on amylose content through grain length to width ratio (-0.307). Grain width indicated a positive indirect effect on amylose content through grain length to width ratio (0.760). Grain length to width ratio expressed a positive indirect effect on amylose content through grain width (0.760) and a negative indirect effect through grain length (-0.307).

Table 3.6: Pearson’s correlation coefficients among agro-morphological characters and grain quality traits

	Traits												
	GL	GW	L/W	ASV	DH	PPP	PH	NETP	PL	PSF	NGP	TGW	GY
AC	0.131	-0.120	0.156	-0.293	0.013	-0.033	0.351*	0.133	-0.122	-0.008	-0.037	-0.020	-0.048
GL		0.062	0.398*	-0.068	-0.158	-0.140	0.184	-0.129	-0.074	-0.013	0.037	0.072	0.053
GW			-0.881**	-0.108	0.203	0.458**	-0.051	0.171	0.110	0.136	-0.103	-0.228	0.159
L/W				0.063	-0.254	-0.449**	0.062	-0.238	-0.127	-0.108	0.090	0.271	-0.092
ASV					0.054	-0.199	0.083	-0.228	0.118	-0.036	-0.034	0.157	-0.064
DH						-0.146	-0.213	-0.110	-0.109	0.034	-0.013	-0.072	-0.102
PPP							-0.292	0.164	0.187	0.186	-0.035	-0.007	0.588**
PH								0.086	-0.048	0.032	-0.074	0.319	-0.215
NETP									-0.009	0.247	0.288	-0.329	0.257
PL										-0.412*	0.144	0.027	0.101
PSF											0.039	0.139	0.358*
NGP												-0.238	0.629**
TGW													0.168

ǂ AC; amylose content; GL, grain length; GW, grain width; L/W, grain length to width ratio; ASV, alkali spreading value; DH, days to heading; PPP, panicles per plant; PH, plant height; NETP, number of effective tillers per plant; PL, panicle length; PSF, percent spikelet fertility; NGP, number of grains per panicle; TGW, weight of 1000 grains; GY, grain yield; *,**, significant at P<0.05, P<0.01 respectively.

Table 3.7: Path coefficient analysis for grain yield with other important agro-morphological characters

	DH	PPP	PH	NETP	PL	PSF	NGP	TGW
DH	**-0.021**	-0.080	0.031	-0.007	0.007	0.005	-0.009	-0.028
PPP	0.003	**0.547**	0.042	0.011	-0.013	0.025	-0.025	-0.003
PH	0.004	-0.159	**-0.146**	0.006	0.003	0.004	-0.053	0.125
NETP	0.002	0.090	-0.013	**0.066**	0.001	0.033	0.206	-0.129
PL	0.002	0.102	0.007	-0.001	**-0.068**	-0.056	0.103	0.010
PSF	-0.001	0.101	-0.005	0.016	0.028	**0.135**	0.028	0.054
NGP	0.000	-0.019	0.011	0.019	-0.010	0.005	**0.715**	-0.093
TGW	0.001	-0.004	-0.046	-0.022	-0.002	0.019	-0.170	**0.391**

ǂDH, days to heading; PPP, panicles per plant; PH, plant height; NETP, number of effective tillers per plant; PL, panicle length; PSF, percent spikelet fertility; NGP, number of grains per panicle; TGW, weight of 1000 grains; Diagonal (bold)=direct path coefficients; Off-diagonal=indirect path values;

Table 3.8: Path coefficient analysis for amylose content with other important grain quality traits

	GL	GW	L/W	ASV
GL	**0.471**	-0.054	-0.307	0.021
GW	0.029	**-0.862**	0.681	0.033
L/W	0.187	0.760	**-0.773**	-0.019
ASV	-0.032	0.093	-0.048	**-0.306**

ᵼL/W, grain length to width ratio; GL, grain length; GW, grain width; AC; amylose content; ASV, alkali spreading value; Diagonal (bold)=direct path coefficients; Off-diagonal=indirect path values;

3.3.4 Population structure

Results of population structure analysis of all upland rice genotypes for *K* ranging from 1 to 10 are shown in Figure 3.3, 3.4 and 3.5. The most significant peak of *ΔK* based on the Evanno et al. (2005) method from STRUCTURE run was observed when *K*=2 (Figure 3.3), which means that the entire panel could be grouped into two major populations based on environmental adaptations and breeding history. Structure analysis further revealed two major clusters as depicted by colour codes (Figure 3.4) indicating genetic relatedness, where genotypes with score >0.80 were considered as pure and <0.80 as admixture. Genotypes obtained from National Crops Resources Research Institute-Uganda (UG) had considerable degree of admixtures. Genotypes from Institut d'Economie Rurale-Mali (IER) and AfricaRice (ARC) represented one cluster, while genotypes from International Centre for Tropical Agriculture (CIAT), Madagascar (MDG) and landraces from South Sudan (LDR) denoted the second gene pool. Using neighbour-joining (NJ) tree method and based on a mean fixation index (F_{st}) estimate value of 0.188, genotypes were grouped into two major clusters (Figure 3.5). Cluster 1 assembled genotypes from ARC, UG and IER, while cluster 2 grouped together genotypes from CIAT, MDG and LDR.

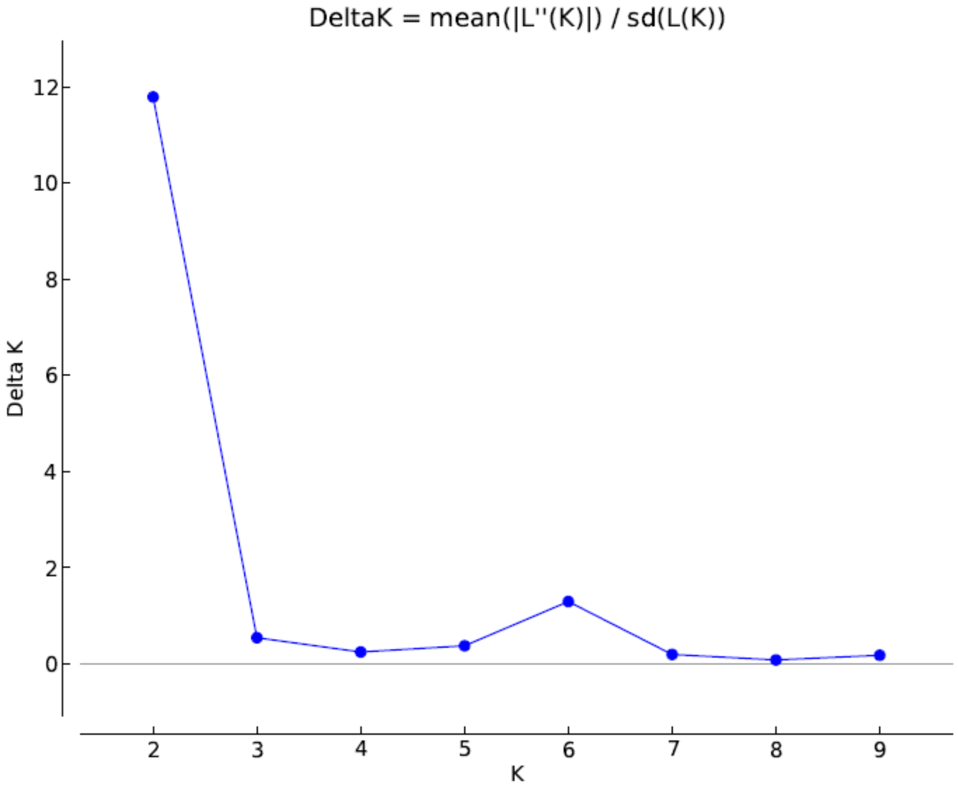


Figure 3.4: Optimum K value of 2 groups established for a panel of 34 elite upland rice genotypes

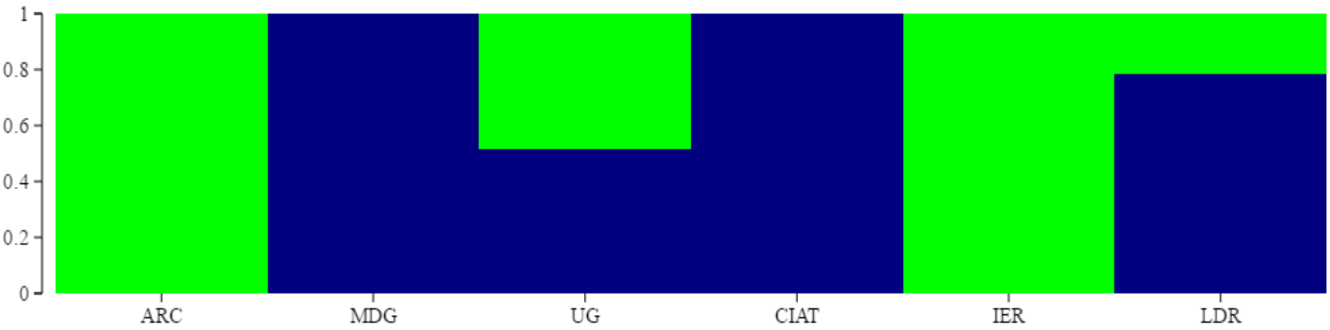


Figure 3.5: Distribution pattern of 34 elite upland rice genotypes based on Bayesian clustering method of DArTseq markers

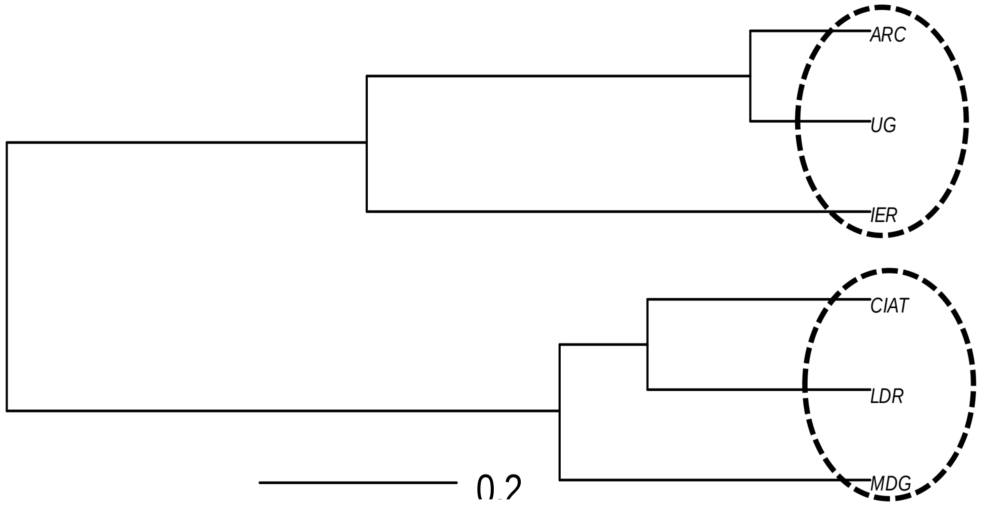


Figure 3.6: Dendrogram of a neighbour-joining (NJ) tree of rice populations constructed for 34 elite upland rice genotypes using DArTseq markers based on a mean fixation index (Fst) estimate value of 0.188

3.3.5 Molecular diversity

Analysis of molecular variance (AMOVA) among 34 rice genotypes (genotypes selected after data filtering process) indicated that 6.7% of the variance was due to genetic differentiation among the populations, 50% of the variance was accounted by genetic differentiation among individuals within populations, while the remaining 43.3% of the variance was due to the differences within individuals (Table 3.9).

Table 3.9: Analysis of molecular variation of a panel of 34 upland rice genotypes

Source of variation	d.f	Sum of squares	Variance components	Percentage variation
Among populations	5	1160.52	7.76	6.71
Among individuals within populations	28	4639.16	57.80	49.98
Within individuals	34	1703.00	50.09	43.31
Total	67	7502.68	115.64	

3.4 Discussion

Genetic variability has been a major driving force in selecting for superior genotypes in crop improvement programmes (Roy et al., 2012), while knowledge on variability and association between yield attributing factors and other important agro-morphological and grain quality traits is critical in tailoring hybridization programmes. Consequently, the efficiency and selection for yield mainly depends on the magnitude and direction of association between yield and other desirable traits such as grain cooking and eating quality traits in rice. In the present study, significant differences were observed for agro-morphological characters, yield components and grain quality traits among 36 upland rice genotypes, indicating the presence of considerable genetic variation among the experimental materials.

Both principal component and cluster analyses suggested substantial variation in the 36 rice genotypes which could be exploited in the breeding programme for enhanced yield and cooking quality. The first and second principal components which cumulatively accounted for 37.2% of variation among the test materials revealed that major contributors to grain yield per plant were number of effective tillers per plant, number of panicles per plant, grain width, number of grains per panicle, panicle length, grain length to width ratio, percent spikelet fertility and weight of 1000 grains. Genotypes in Cluster 2 including; ‘P5H6’, ‘NAMCHE 6’ and ‘ART3-7L9P8-3-5-B-B-2’ had considerable high yields and intermediate values for AC and ASV (which relates to intermediate GT) and could be considered potential candidates for breeding or direct production in South Sudan. According to Akram (2009), high quality rice is strikingly characterized by intermediate AC, intermediate GT, soft gel consistency (GC), strong aroma and a high degree of grain elongation. Additionally, AC is considered to be one of the most important indirect indices of rice cooking, eating and processing quality (Ni et al., 2011; Asghar et al., 2012), since it determines the hardness of cooked rice, gloss of the final product and rice-water ratio.

Correlation coefficients analysis revealed that grain yield had significant positive correlation with number of panicles per plant, percent spikelet fertility and number of grains per panicle. This suggests the importance of these characters for yield improvement. Similar results were observed by other workers (Akhtar et al., 2011; Xu et al., 2015) for number of grains per panicle and by Shinde et al. (2015), Abdala et al. (2016) and Ratna et al. (2015) for number of panicles per plant and percent spikelet fertility. Yield being a complex trait influenced by various yield contributing factors and the environment (Ratna et al., 2015), selection based on yield alone can be misleading (Shinde et al., 2015), thus an effective selection strategy should incorporate the association between yield and its attributing factors.

Path coefficient analysis provides a better understanding of yield contributing characters by partitioning the correlation co-efficient into components of direct and indirect effects (Ratna et al., 2015). In the present study, number of grains per panicle exerted the highest direct positive effect on grain yield per plant and also indicated a significant positive genotypic correlation with the same. This implies that direct selection for this trait would be much more effective for enhancing grain yield in upland rice. Several other workers reported similar findings (Zahid et al., 2006; Khan et al., 2009; Rashid et al., 2013; Pham et al., 2016).

Other agro-morphological traits such as number of panicles per plant, weight of 1000 grains and percent spikelet fertility also exhibited positive direct effect on grain yield per plant, hence direct selection of these traits will accordingly contribute to enhanced upland rice grain yield. Seyoum et al. (2012) and (Rai et al., 2015) showed that number of panicles per plant exerted positive direct effect on grain yield per plant. Chaudhary et al. (2016) and Singh et al. (2016) found positive direct effect of weight of 1000 grains on grain yield per plant. Furthermore, Naseer et al. (2015) also reported positive direct effect of percent spikelet fertility on grain yield per plant. In addition, plant height exhibited the largest negative direct effect on grain yield. Thus selecting for this trait will be undesirable. Rai et al. (2015) and Hairmansis et al. (2013) also reported similar findings.

Grain length exhibited the greatest maximum direct positive effect on amylose content, while grain width revealed the largest negative effect on the same. Furthermore, AC was positively correlated with grain length and grain length to width ratio but negatively correlated with grain width and alkali spreading value. In another study, Ge et al. (2008) indicated that AC of rice was positively correlated with grain length and width but negatively correlated with grain length to width ratio. Roy et al. (2012) suggested that selection for long grain with slender shape will simultaneously increase AC and GT. These relationships suggest that selection for longer and slender grains could result in some concurrent increase in AC.

Success of any crop improvement programme depends on the amount of genetic diversity and the extent to which the desirable characters are heritable (Ravi et al., 2003). Furthermore, genetic characterization of rice germplasm encourages utilization by rice breeding programmes for rice improvement. Diversity Arrays Technology (DArT) markers have been used in the construction of high-density maps, quantitative trait loci (QTL) mapping and genetic diversity analysis because of their efficiency and low cost (Gupta et al., 2008). In the current study, using DArTseq-derived SNP markers for structure analysis, 34 upland rice genotypes were separated into 2 major populations representing two major gene pools. Admixtures were also revealed in genotypes of UG origin suggesting considerable level of interbreeding between populations due to the local breeding activities. The level of admixtures may also suggest the origin of those individuals with unknown population characteristics. In addition, a low mean fixation index (F_{st}) estimate value of 0.188 indicated a considerable low degree of differentiation among the populations. This was further revealed by analysis of molecular variance (AMOVA) which indicated that 6.7% of the variance was due to genetic differentiation among the populations. In addition, the neighbour-joining tree method grouped populations that show close genetic similarities into two major clusters based on environmental adaptations and breeding history. Populations in Cluster 1 (ARC, UG and IER) are closely related possibly due to rice germplasm exchange given the similarities in environmental adaptation and pedigree information. Similarly, Cluster 2 populations (CIAT, LDR and MDG) suggested genetic similarities probably due to movement of rice materials and comparable agro-ecological environments for rice genotypes cultivated within CIAT and MDG populations. Genotypes within the LDR population suggested admixtures which may have been generated from interbreeding activities aimed at introgressing favourable alleles from the MDG population.

3.5 Conclusion

In the present study, considerable variations were observed for agro-morphological and grain quality traits among the tested rice genotypes. Furthermore, molecular characterization of test materials revealed 2 distinct clusters suggesting genetic relatedness. However a relatively low mean fixation index (F_{st}) estimate value with increased frequency of admixtures as a consequence of local breeding activities was suggestive of low degree of differentiation among populations. Thus, it will be important to broaden the genetic base of the current rice genotype collection by introducing distant or wild relatives with potential for novel genes or quantitative trait loci for important agro-morphological and grain quality traits. Plausible associations identified for yield and grain quality attributing factors could be considered important selection criteria for enhancing yield and grain quality in rice. In addition, selection of high yielding upland rice varieties with intermediate AC and GT values are potential candidate varieties for introduction in South Sudan. The study further suggests the use of number of grains per panicle, number of panicles per plant, weight of 1000 grains, percent spikelet fertility and grain length as essential selection indices for genetic improvement of yield and cooking quality trait in rice. Overall, using DArTseq markers and agro-morphological and grain quality traits the following genetically diverse rice genotypes: ‘P5H6’, ‘NAMCHE 6’ and ‘ART3-7L9P8-3-5-B-B-2’ were selected for direct production or use in breeding.

References

Abdala, A., J. Bokosi, A. Mwangwela and T. Mzengeza. 2016. Correlation and path co-efficient analysis for grain quality traits in F1 generation of rice (*Oryza sativa* L.). Journal of Plant Breeding and Crop Science 8: 109-116.

AfDB. 2013. African Development Bank Group.South Sudan: An Infrastructure Action Plan - A Program for Sustained Strong Economic Growth. pp. 131-167.

Akhtar, N., M. Nazir, A. Rabnawaz, T. Mahmood, M. Safdar, M. Asif and A. Rehman. 2011. Estimation of heritability, correlation and path coefficient analysis in fine grain rice (*Oryza sativa* L.). The Journal of Animal and Plant Sciences. 21: 660-664.

Akhtar, S., M. Bhat, S.A. Wani, K. Bhat, S. Chalkoo, M. Mir and S.A. Wani. 2010. Marker Assisted Selection in Rice. Journal of Phytology. 2(10).

Akram, M. 2009. Aromatic rices of Pakistan-a review. Pakistan Journal of Agricultural Research 22: 154-160.

Anuonye, J., O. Daramola, C. Chinma and O. Banso. 2016. Effects of processing methods on physicochemical, functional and sensory properties of Ofada rice. International Journal of Biotechnology and Food Science. 4(1): 7-14.

Asghar, S., F.M. Anjum, R.M. Amir and M.A. Khan. 2012. Cooking and eating characteristics or rice (*Oryza sativa* L.). Pakistan Journal of Food Sciences. 22(3): 128-132.

Balakrishnan, D., S. Robin, R. Rabindran and A. Joel. 2016. Molecular and Morphological Characterisation of Back Cross Generations for Yield and Blast Resistance in Rice. Journal of Rice Research. 4(169): 2.

Borba, T.C.d.O., R.P.V. Brondani, F. Breseghello, A.S.G. Coelho, J.A. Mendonça, P.H.N. Rangel and C. Brondani. 2010. Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.). Genetics and Molecular Biology 33(3): 515-524.

Chaudhary, A., K. Srivastava, V. Agrawal and S. Kumar. 2016. Elucidation of Variability, Interrelationships and Path-coefficient in Maize (*Zea mays L*.). Nature Environment and Pollution Technology. An International Quarterly Scientific Journal. 15(2): 653-656.

Concepcion, J.C.T., M. Ouk, D. Zhao and M.A. Fitzgerald. 2015. The need for new tools and investment to improve the accuracy of selecting for grain quality in rice. Field Crops Research 182: 60-67.

Cruz, N.D. and G. Khush. 2000. Rice grain quality evaluation procedures. Aromatic Rices 3: 15-28.

Dewey, D.R. and K.H. Lu. 1959. A correlation and path analysis of components of crested wheat grass seed production. Agronomy Journal 51: 515-518.

Dhurai, S.Y., D.M. Reddy and S. Ravi. 2016. Correlation and Path Analysis for Yield and Quality Characters in Rice (*Oryza sativa* L.). Rice Genomics and Genetics 7(4): 1-6.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. Plos One 6: e19379.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14(8): 2611-2620.

Fitzgerald, M.A., S.R. McCouch and R.D. Hall. 2009. Not just a grain of rice: the quest for quality. Trends in Plant Science. 14: 133-139.

Ge, G., C. Shi, J. Wu and Z. Ye. 2008. Analysis of the genetic relationships from different genetic systems between the amylose content and the appearance quality of indica rice across environments. Genetics and Molecular Biology 31: 711-716.

Gibson, T., V. Solah and B. McCleary. 1997. A procedure to measure amylose in cereal starches and flours with concanavalin A. Journal of Cereal Science 25: 111-119.

Govindaraj, P., K. Vinod, S. Arumugachamy and M. Maheswaran. 2009. Analysing genetic control of cooked grain traits and gelatinization temperature in a double haploid population of rice by quantitative trait loci mapping. Euphytica 166: 165-176.

Gupta, P., S. Rustgi and R. Mir. 2008. Array-based high-throughput DNA markers for crop improvement. Heredity 101: 5-18.

Hairmansis, A., B. Kustianto and S. Suwarno. 2013. Correlation analysis of agronomic characters and grain yield of rice for tidal swamp areas. Indonesian Journal of Agricultural Science 11: 11-15.

He, J., X. Zhao, A. Laroche, Z.X. Lu, H. Liu and Z. Li. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in Plant Science. 5: 484.

Hossain, M.I., N.M.F. Rahman, M.S. Kabir, M.A.B. Siddique and M. Tareq. 2015. Development and Validation of Producer and Consumer Preference Models for Rice Varieties in Bangladesh. Bangladesh Rice Journal 19: 63-71.

IBM, C. 2012. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

IRRI. 2002. The standard evaluation system for rice reference manual. International Rice Research Institute, Los Banos, Philippines. doi:www.knowledgebank.irri.org/extension/index.php/ses.

Juliano, B. 1971. A simplified assay for milled-rice amylose. Cereal Science Today 16: 334-360.

Khan, A.S., M. Imran and M. Ashfaq. 2009. Estimation of genetic variability and correlation for grain yield components in rice (*Oryza sativa* L.). American-Eurasian Journal of Agricultural and Environmental Science. 6(5): 585-590.

Little, R.R., G.B. Hilder and E.H. Dawson. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry. 35(2): 111-126. .

Masette, M., A. Candia, E. Khakasa, S. Okurut and S. Tinyiro. 2015. Preferences of Ugandan consumers for rice varieties and brands on the local market. Uganda Journal of Agricultural Sciences 14: 1-11.

Morrison, W.R. and B. Laignelet. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. Journal of Cereal Science 1: 9-20.

Naseer, S., M. Kashif, H.M. Ahmad, M.S. Iqbal and Q. Ali. 2015. Estimation of genetic association among yield contributing traits in aromatic and non-aromatic rice (*Oryza sativa* L.) cultivars. Life Science Journal. 12(4): 68-73.

Ni, D., S. Zhang, S. Chen, Y. Xu, L. Li, H. Li, Z. Wang, X. Cai, et al. 2011. Improving cooking and eating quality of Xieyou57, an elite indica hybrid rice, by marker-assisted selection of the Wx locus. Euphytica 179: 355-362.

Nzomoi, J. and I. Anderson. 2013. The Rice Market in East Africa. Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists, September 22-25, 2013,Hammamet,Tunisia.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Resources 6: 288-295.

Pham, H.T.T., K.T. Do, M.N. Truong, X.D. Tran, L.T. Nguyen and B.C. Bui. 2016. Path analysis for yield traits in F2 generation and molecular approaches for breeding rice tolerant to drought and submergence. African Journal of Agricultural Research 11: 2329-2336.

Phing, W.C., M.A. Latif, M. Y. Rafii, M.R. Ismail and A. Puteh. 2016. Advances to improve the eating and cooking qualities of rice by marker-assisted breeding. Critical reviews in biotechnology 36: 87-98.

Poland, J.A. and T.W. Rife. 2012. Genotyping-by-sequencing for plant breeding and genetics. The Plant Genome 5: 92-102.

Premkumar, R., R. Gnanamalar and C. Anandakumar. 2016. Correlation and path coefficient analysis of grain quality traits in Rice (*Oryza sativa* L.). Indian Journal Of Agricultural Research. 50 (1): 27-32.

Pritchard, J.K., M. Stephens, N.A. Rosenberg and P. Donnelly. 2000. Association mapping in structured populations. The American Journal of Human Genetics 67: 170-181.

Pritchard, J.K., W. Wen and D. Falush. 2003. Documentation for structure software: version 2.

Rai, P., U. Sarker, P. Roy and A. Islam. 2015. Character association in F4 generation of rice (*Oryza sativa* L.). Bangladesh Journal of Plant Breeding and Genetics 26: 39-44.

Ramasamy, R.K., S. Ramasamy, B.B. Bindroo and V.G. Naik. 2014. STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3: 431.

Rashid, K., I. Khaliq, M. Farooq and M. Ahsan. 2013. Correlation and Cluster Analysis of Some Yield and Yield Related Traits in Rice (*Oryza sativa* L.). International Journal of Agricultural Science Research. 3(4): 25-30.

Ratna, M., S. Begum, A. Husna, S. Dey and M. Hossain. 2015. Correlation and path coefficients analyses in Basmati rice. Bangladesh Journal of Agricultural Research 40: 153-161.

Ravi, M., S. Geethanjali, F. Sameeyafarheen and M. Maheswaran. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica. 133(2): 243-252.

Roy, S., A. Banerjee, B.K. Senapati and G. Sarkar. 2012. Comparative analysis of agro-morphology, grain quality and aroma traits of traditional and Basmati-type genotypes of rice, *Oryza sativa* L. Plant Breeding. 131(4): 486-492.

Saha, P.S., M.U. Islam, M.S. Islam and M.A. Salam. 2016. Analysis of Yield Components and Aroma of Small Grain Aromatic Rice (*Oryza sativa* L.) in Bangladesh. The Agriculturists. 13(2): 17-24.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4): 406-425.

Sánchez-Sevilla, J.F., A. Horvath, M.A. Botella, A. Gaston, K. Folta, A. Kilian, B. Denoyes and I. Amaya. 2015. Diversity Arrays Technology (DArT) marker platforms for diversity analysis and linkage mapping in a complex crop, the octoploid cultivated strawberry (Fragaria× ananassa). Plos One 10: e0144960.

Seyoum, M., S. Alamerew and K. Bantte. 2012. Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice (*Oryza sativa* L.). Journal of plant sciences. 7(1): 13.

Shinde, S., K. Kumar and R. Pawar. 2015. Genetic studies in upland rice (*Oryza sativa* L.). International Journal of Plant Sciences (Muzaffarnagar). 10(1): 33-37.

Singh, M. Punia and V. Singh. 2016. Inter-relationship between grain yield and its component characters in F2 generation of bread wheat (*Triticum aestivum* L.). International Journal of Current Advanced Research. 5(4): 749-751.

Singh, R. and B. Chaudhary. 1977. Variance and covariance analysis. Biometrical methods in quantitative genetics analysis. Revised edition. Kalyani, Ludhiana, New Delhi, India. 39-69.

Vanaja, T. and T.B. Luckins. 2006. Variability in grain quality attributes of high yielding rice varieties (*Oryza sativa* L.) of diverse origin. Journal of Tropical Agriculture. 44(1-2): 61-63.

VSN, I. 2011. GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

Wang, P., Y. Xing, Z. Li and S. Yu. 2012. Improving rice yield and quality by QTL pyramiding. Molecular Breeding 29: 903-913.

Xu, Q., W. Chen and Z. Xu. 2015. Relationship between grain yield and quality in rice germplasms grown across different growing areas. Breeding science 65: 226.

Yi, M., K.T. Nwe, A. Vanavichit, W. Chai-arree and T. Toojinda. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113: 178-186.

Yun, S.H. and N.K. Matheson. 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-a. Starch-Stärke 42: 302-305.

Zahid, M.A., M. Akhter, M. Sabar, Z. Manzoor and T. Awan. 2006. Correlation and path analysis studies of yield and economic traits in Basmati rice (*Oryza sativa* L.). Asian Journal of Plant Science. 5(4): 643-645.

4. Diversity analysis and genome-wide association studies of grain quality traits in rice (*Oryza sativa* L.) using DArT markers

Abstract

Microarray-based markers such as Diversity Arrays Technology (DArT) have become the genetic markers of choice for construction of high-density maps, quantitative trait loci (QTL) mapping and genetic diversity analysis based on their efficiency and low cost. More recently, the DArT technology was further developed in combination with high-throughput next-generation sequencing (NGS) technologies to generate the DArTseq platform representing a new sequencing tool of complexity-reduced representations. In this study, DArTseq markers were used to determine genetic diversity and genome-wide association studies (GWAS) of grain quality traits in rice (*Oryza sativa* L.). The study was performed using 59 rice genotypes with 525 SNPs derived from DArTseq platform. Population structure analysis revealed only two distinct genetic clusters where genotypes were grouped based on environmental adaptation and pedigree information. Analysis of molecular variance indicated a low degree of differentiation among populations suggesting the need for broadening the genetic base of the current germplasm collection. GWAS revealed 22 significant associations between DArTseq-derived SNP markers and rice grain quality traits in the test genotypes. Two of the 22 associations were in chromosomal regions where the QTLs associated with the given traits had previously been identified, while the remaining 20 significant SNP marker loci were indicative of the likelihood discovery of novel alleles associated with rice grain quality traits. DArTseq-derived SNP markers that include SNP12_100006178, SNP13_3052560 and SNP14_3057360 individually co-localised with two functional gene groups that were associated with QTLs for grain width and grain length to width ratio on chromosome 3, indicating trait dependency or pleiotropic-effect loci. This study demonstrated that DArTseq markers were useful genomic resources for genome-wide association studies of rice grain quality traits to accelerate varietal development and release.

Keywords: Diversity Arrays Technology, Genome-wide association studies, Genotyping by sequencing, Grain quality, *Oryza sativa* L.

4.1 Introduction

Rice (*Oryza sativa* L.) is increasingly becoming a major food crop in sub-Saharan Africa (SSA). Globally, rice is one of the most widely cultivated cereal crops distributed across diverse geographical, ecological and climatic conditions (Traoré et al., 2015; Malathi et al., 2016). Given the varied adaptations of rice genotypes, several accessions are available with wide phenotypic and genotypic diversity (Das et al., 2013). A great number of these rice accessions, belonging to different sub-species including *indica*, *japonica* and *javanica*, have been conserved in global gene banks (Garris et al., 2005). This is important as a potential source of reservoir genes that could be exploited in crop improvement programmes (Brar and Khush, 1997; Koutroubas et al., 2004). However, only a slight amount of the available rice genetic resources have been utilized in most rice breeding programmes (Malathi et al., 2016), hence a great genetic similarity exists in most commercial rice cultivars given the narrow genetic base (Das et al., 2013).

Most rice breeding programmes in SSA face the challenge of improving not only the yield potential but also other important grain quality traits such as cooking and processing qualities (Asghar et al., 2012; Demont, 2013; Malathi et al., 2016). Furthermore, grain quality and in particular cooking and eating quality always represents a major criterion in evaluating rice grain quality (Wang et al., 2010). Rice cooking and eating quality is strongly determined by the level of amylose content (AC) (Dobo et al., 2010; Biselli et al., 2014), where high AC in the endosperm is usually associated with dry, fluffy, and separated cooked rice grains, and represents the key determinant of poor cooking and eating quality (Juliano, 1985). In addition, rice grain shape is an important character which subsequently affects cooking quality (Li et al., 2004a; Qiu et al., 2012). Rice grain shape is determined by its three dimensions including, grain length (GL), grain width (GW) and grain length to width ratio (L/W).

The genetic basis of rice grain shape has been well studied (Xiao et al., 1998; Jiang et al., 2005) and several quantitative trait loci (QTLs) underlying grain shape have been detected and fine mapped (Bai et al., 2010; Zhang et al., 2012). However, the identified QTLs may not be sufficient to elucidate the genetic basis of rice grain shape. Furthermore, the varied nature of rice grain shape underscores the need for identifying novel QTLs in order to design a breeding strategy for grain shape improvement. Thus, knowledge of AC and its associated traits represents a major criterion in developing rice cultivars with desirable cooking and eating quality (Wang et al., 2010).

In addition, it is essential to broaden the genetic base of rice genotypes by introducing genes from distant or wild relatives with potential for delivering novel genes or quantitative trait loci (QTLs) for important agronomic traits. The magnitude of genetic variability and the extent to which the desirable characters are heritable largely determines the success of any plant breeding programme (Vanaja and Luckins, 2006). Consequently, association mapping (AM) based on phenotypic and genotypic data has been critical in identifying molecular markers or QTLs linked to traits of interest and with potential for use in marker-assisted selection (MAS). This has allowed the use of diverse set of germplasm that provides a broader allelic coverage without necessarily developing bi-parental mapping populations (Tadesse et al., 2015).

More recently with the advances in next generation sequencing (NGS) technologies, genotyping-by-sequencing (GBS) has emerged as a promising genomic approach for simultaneous exploration of plant genetic diversity and molecular marker discovery (Elshire et al., 2011; Poland and Rife, 2012; He et al., 2014). Thus, GBS has effectively been used for single-nucleotide polymorphisms (SNP) marker discovery and QTL identification of tightly linked marker-trait associations (Boutet et al., 2016; Tang et al., 2016) and in the application of genomic selection of complex traits for crop improvement (Jarquín et al., 2014; Furuta et al., 2017). The GBS approach is therefore considered an important cost-effective tool for population genetics, QTL discovery, high-resolution mapping and for genomic selection in plant breeding programmes (He et al., 2014; Furuta et al., 2017).

With advances in microarray-based marker technology, Diversity Arrays Technology (DArT) markers have become the genetic markers of choice for construction of high-density maps, mapping quantitative trait loci (QTL) and genetic diversity analysis based on their efficiency and low cost (Gupta et al., 2008). Additionally, by combining the complexity reduction of the DArT method with high-throughput next-generation sequencing (NGS) technologies, the DArTseq platform was developed signifying a new implementation of sequencing of complexity reduced representations (Sánchez-Sevilla et al., 2015). Consequently, DArTseq markers based on GBS technology have been successfully applied for linkage mapping, QTL identification in bi-parental mapping population, genome wide association studies (GWAS), genetic diversity, as well as in marker-assisted and genomic selection (Sánchez-Sevilla et al., 2015). Hence, DArTseq marker platform has been widely applied (Kilian et al., 2012; Courtois et al., 2013; Von Mark et al., 2013) and is rapidly gaining popularity as a preferred method of genotyping-by-sequencing (Sánchez-Sevilla et al., 2015). The objective of this study was to determine genetic diversity and genome-wide association studies (GWAS) of grain quality traits in a diverse collection of 59 upland and lowland rice (*Oryza sativa* L.) genotypes.

4.2 Materials and methods

4.2.1 Germplasm and phenotyping

The present study used a collection of 59 rice genotypes, which included 3 popular landraces, 36 upland and 22 lowland rice collections (Table 4.1). The sampled collections were introductions from the International Rice Research Institute (IRRI), AfricaRice (ARC), National Crops Resources

Research Institute (NaCRRI-Uganda), International Centre for Tropical Agriculture (CIAT), Madagascar (MDG), Tanzania (TZ) and Institut d'Economie Rurale (IER-Mali), while the landraces (LDR) were collections from South Sudan. The study was conducted at the Biosciences East and Central Africa (BeCA-ILRI), Nairobi, Kenya. Test materials were assessed for determinants of grain quality (grain shape, amylose content, and alkali spreading value) using dehusked grains. Grain shape was classified on the basis of grain length (GL), grain width (GW) and length to width ratio (L/W), where measurements were read using a vernier calliper as described by Cruz and Khush (2000).

Table 4.1: List of rice genotypes used in the study

Entry No.	Name/pedigree	Ecology	Origin	PID ł
1	GSR-I-0057	Lowland	ARC	ARC
2	K 5	Lowland	NaCRRI	UG
3	WAC116X NERICA 4	Lowland	Mali	IER
4	NERICA L 19	Lowland	ARC	ARC
5	K-85	Lowland	NaCRRI	UG
6	JARIBU	Lowland	Tanzania	TZ
7	TAI	Lowland	IRRI	IRRI
8	K85-10	Lowland	NaCRRI	UG
9	KOMBOKA	Lowland	IRRI	ARC
10	1052 SUPA LINE	Lowland	IRRI	IRRI
11	K 38	Lowland	NaCRRI	UG
12	TXD 306	Lowland	ARC	ARC
13	WITA 9	Lowland	ARC	ARC
14	NERICA 6	Lowland	ARC	ARC
15	1189 LINE	Lowland	ARC	ARC
16	1191 LINE	Lowland	ARC	ARC
17	326104 LINE	Lowland	ARC	KR
18	Supa TZ	Lowland	Tanzania	TZ
19	Basmati 370	Lowland	IRRI	IRRI
20	SK-95-4	Lowland	Mali	IER
21	SK-7-8	Lowland	Mali	IER
22	BR4	Lowland	Landrace	LDR
23	BG 400-1	Lowland	Landrace	LDR
24	NAMCHE 6	Upland	NaCRRI	UG
25	NAMCHE 1	Upland	NaCRRI	UG
26	NAMCHE 3	Upland	NaCRRI	UG
27	NAMCHE 2	Upland	NaCRRI	UG
28	Namche 4	Upland	NaCRRI	UG
29	Namche 5	Upland	NaCRRI	UG
30	SCRIDO 37-4-2-2-5	Upland	Madagascar	MDG
31	P5 H12	Upland	NaCRRI	UG
32	P24 H10	Upland	NaCRRI	UG
33	CT11891-3-3-3-M-1-2-2-M	Upland	CIAT	CIAT
34	P5 H6	Upland	NaCRRI	UG
35	ART12-L4P7-21-4-B-3	Upland	ARC	ARC
36	ART10-1L15P1-4-3-1	Upland	ARC	ARC
37	ART2-4L3P1-2-1	Upland	ARC	ARC
38	SCRIDO 06-2-4-3-4-5	Upland	Madagascar	MDG
39	ART3 -8L6P3-2-3-B	Upland	ARC	ARC
40	P27 H4	Upland	NaCRRI	UG
41	P26 H1	Upland	NaCRRI	UG
42	ART3-7L9P8-3-5-B-B-2	Upland	ARC	ARC
43	P5 H14	Upland	NaCRRI	UG
44	P27 H3	Upland	NaCRRI	UG
45	ART3 -7L3P3-B-B-2	Upland	ARC	ARC
46	P23 H1	Upland	NaCRRI	UG
47	ART3-8L6P3-2-3-B	Upland	ARC	ARC
48	Mbume	Upland	Landrace	LDR
49	ART25-3-29-2-B	Upland	ARC	ARC
50	ART3-8L6P3-2-2-B	Upland	NaCRRI	UG
51	ART12-L2P2-20-3-1-1	Upland	ARC	ARC
52	P24 H1	Upland	ARC	ARC
53	P62 H17	Upland	NaCRRI	UG
54	ART16-4-11-13-4	Upland	NaCRRI	UG
55	PCT-4\0\0\0>19-M-1-1-5-1-M	Upland	ARC	ARC
56	NERICA 4	Upland	ARC	ARC
57	DKAP-27	Upland	Mali	IER
58	NERICA 1	Upland	ARC	ARC
59	NERICA 10	Upland	ARC	ARC

ł PID=Population Identity; IRRI, International Rice Research Institute; ARC, AfricaRice; UG, National Crops Resources Research Institute(NaCRRI)-Uganda; IER, Institut d'Economie Rurale – Mali, CIAT, International Centre for Tropical Agriculture ; MDG, Madagascar ;TZ, Tanzania ;LDR, Landrace-South Sudan;

4.2.2 Quantification of amylose and amylopectin

Amylose and amylopectin content of the starch were determined by the method of Gibson et al. (1997) as described in chapter 3, section 3.3.3.

4.2.3 Measurement of gelatinization temperature

Gelatinization temperature (GT) was assessed indirectly as the alkali spreading value of hulled kernels as per modified procedure of Little et al. (1958) as referred in chapter 3, section 3.3.4.

4.2.4 DNA isolation and genotyping

Total genomic DNA was isolated from leaves of three-week old plants using the ZYMO research *Quick*-DNA™ Plant/Seed 96 Kit, where a single individual plant was considered for each genotype. Subsequently, 40 µl of a 50 ng/µl DNA of each sample were sent to Diversity Arrays Technology (DArT) Pty Ltd, Australia ('http://www.diversityarrays.com/dart-map-sequences') for whole genome scan using Diversity Arrays Technology (DArT) markers. Whole-genome genotyping for the 59 rice genotypes was carried out using Genotyping-By-Sequencing (GBS) technology as described by Elshire et al. (2011) using 18,927 DArT markers. The markers were integrated into a linkage map by inferring marker order and position from the consensus DArT map.

4.2.5 Data filtering process and DArTseq SNP calling

DArTseq-derived SNP markers were filtered to remove bad SNPs and genotypes using PLINK 1.9 software in MS window and R statistical software, where genotypes with > 30% missing data, SNP loci with >20% missing data (Figure 4.1) and rare SNPs with <5% minor allele frequencies (MAF) were pruned. Only 525 DArTseq informative SNPs and 59 genotypes were considered after filtering and data quality control process.

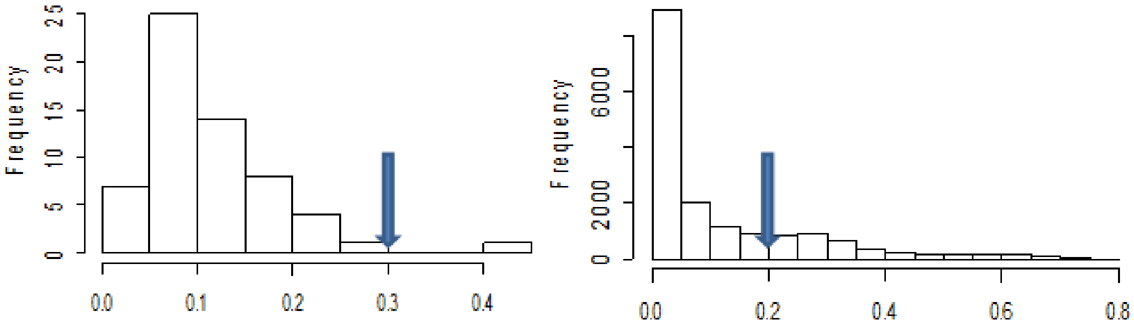


Figure 4.1: Frequency of genotypes with missing data (left); frequency of SNPs (loci) with missing data (right)

4.2.6 Data analysis

4.2.6.1 Population structure analysis

The genetic structure and relationship among 59 rice genotypes was investigated using 525 DArTseq-derived SNP markers distributed across the rice genome as described by Pritchard et al. (2000). Bayesian clustering method was applied to identify clusters of genetically similar individuals using the software STRUCTURE version 2.3 (Pritchard et al., 2003). Cluster values (K) ranging from 1 to 10, and ten independent runs were used for each value in order to obtain consistent results. The best K-value for estimating a suitable population size for the dataset was determined as K=2 based on the Evanno et al. (2005) method from STRUCTURE run. In addition, population differentiation due to genetic structure was assessed using a neighbour-joining (NJ) tree method (Saitou and Nei, 1987) and Principal Component Analysis (PCA) generated by R statistical software. Analysis of molecular variance (AMOVA) and genetic diversity was performed using GenAlEx V6.5 software (Peakall and Smouse, 2006). DArTseq-derived SNP data were numerically coded as follows: A= 1, C= 2, T= 3, G= 4 and missing data was coded as 0 as suggested in GenAlEx V6.5 user manual.

4.2.6.2 Linkage disequilibrium

Linkage disequilibrium analysis was performed using TASSEL V5.3.1 software (Bradbury et al., 2007) with selected 525 DArTseq-derived SNP markers of known position (Huang et al., 2012) out of the complete set of 18,927 polymorphic markers. Linkage disequilibrium was estimated as squared allele frequency correlations (R^2), and only P-values ≤0.01 for each pair of loci were considered significant.

4.2.6.3 Association mapping

Determinants of grain quality including grain length (GL), grain width (GW), grain length/width ratio (L/W), amylose content (AC) and gelatinization temperature (GT) were considered for association mapping. Association mapping analysis was performed with TASSEL V5.3.1 software (Bradbury et al., 2007) using both the General Linear Model (GLM) and Mixed Linear Model (MLM) methods. Two different methods were considered for both GLM and MLM; where, for GLM, the model with no control for population structure and relatedness (naive model), and the model with population structure (the Q model) were performed, whereas for MLM; the model that considers the familial relatedness between accessions (the K model), and the model that takes into account both the population structure and the familial relatedness were used, that is, Q + K model as described by Yu et al. (2006). Where, the general equations for GLM and MLM are: $y = Xa + e$; and $y = Xa+ Qb+ Zu + e$, respectively; where, y is vector for phenotypes, a is the vector of marker fixed effects, b is a vector of fixed effects, u is the vector of random effects, and e is the vector of residuals. X denotes the genotypes at the marker; Q is the Q-matrix obtained from the STRUCTURE software and Z is an identity matrix. Both models were applied with and without considering the fixed effect of the population structure. Marker alleles with P-values ≤0.001 in both MLM and MLM-Q models were declared significantly associated with grain quality parameters.

4.3 Results

4.3.1 Genetic diversity

The number of accessions, number of alleles, genetic diversity, heterozygosity, polymorphism information content (PIC) and major allele frequency of the eight populations is shown in Table 4.2. The mean PIC values for each SNP locus in rice collections from ARC, CIAT, IER, IRRI, LDR, MDG, TZ and UG were 0.34, 0.02, 0.27, 0.29, 0.23, 0.10, 0.06 and 0.34, respectively. The mean number of alleles for each population was 2.0, 1.05, 1.94, 1.90, 1.77, 1.28, 1.19 and 2.0 respectively. The magnitudes of PIC and mean number of alleles were in the order ARC=UG > IRRI > IER > LDR > MDG > TZ > CIAT. Rice population from ARC had the highest level of PIC, gene diversity and mean number of allele, but lowest level of major allele frequency (0.64). Rice population from CIAT had the lowest level of PIC, gene diversity and mean number of allele, but the highest level of major allele frequency (0.98).

Table 4.2: Estimation of gene diversity, heterozygosity, PIC and major allele frequency in 59 rice accessions

Group	No. of accessions	Allele.No	Gene Diversity	Heterozygosity	ł PIC	Major Allele Frequency
ARC	22.00	2.00	0.45	0.09	0.34	0.64
CIAT	1.00	1.05	0.02	0.05	0.02	0.98
IER	4.00	1.94	0.34	0.08	0.27	0.76
IRRI	4.00	1.90	0.37	0.07	0.29	0.71
LDR	3.00	1.77	0.29	0.20	0.23	0.78
MDG	2.00	1.28	0.14	0.06	0.10	0.87
TZ	2.00	1.19	0.08	0.09	0.06	0.94
UG	21.00	2.00	0.44	0.14	0.34	0.66

ARC, AfricaRice; CIAT, International Centre for Tropical Agriculture; IER, Institut d'Economie Rurale–Mali; IRRI, International Rice Research Institute; LDR, Landrace-South Sudan; MDG, Madagascar; TZ, Tanzania; UG, National Crops Resources Research Institute-Uganda (NaCRRI); ł Polymorphism information content

4.3.2 Population structure and genetic relationships

Results of population structure analysis of 59 rice genotypes using a model-based programme, STRUCTURE, for *K* ranging from 1 to 10, and by inferring on Delta K of Evanno et al. (2005) identified the most suitable K value for determining the genetic cluster as K=2 (Figure 4.2). The number of populations were visualized using Structure Plot V2.0 (Ramasamy et al., 2014), where genotypes that scored >0.80 were considered as pure and <0.80 as admixture (Figure 4.3). Only genotypes with origin from the National Crops Resources Research Institute-Uganda (UG) suggested considerable degree of admixtures (<80%). Two major clusters were formed where genotypes from UG, AfricaRice (ARC), Madagascar (MDG) and International Centre for Tropical Agriculture (CIAT) formed the first cluster, while genotypes from International Rice Research Institute (IRRI), Tanzania (TZ), Institut d'Economie Rurale-Mali (IER) and landraces from South Sudan (LDR) comprised the second cluster. Similarly, using Neighbour Joining (NJ) method and based on a mean fixation index (F_{st}) estimate value of 0.134 generated by PLINK 1.9 software, genotypes were grouped into two major clusters (Figure 4.4), confirming the results of population structure analysis. Cluster 1 assembled genotypes from UG, ARC, MDG and CIAT, while cluster 2 grouped together genotypes from IRRI, TZ, IER and LDR.

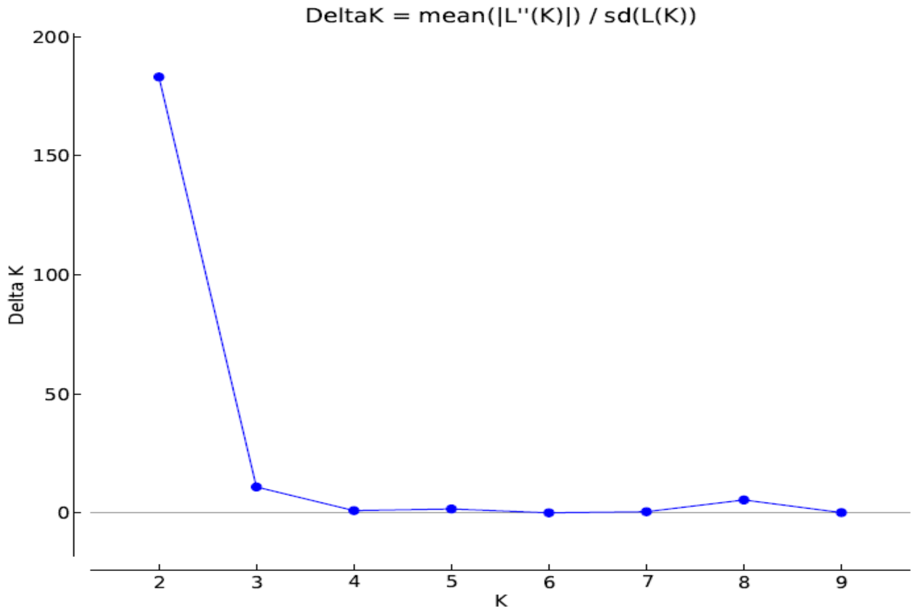


Figure 4.2: Magnitude of Δ K as a function of Delta K for 59 rice genotypes based on 525 polymorphic DArTseq-derived SNP markers

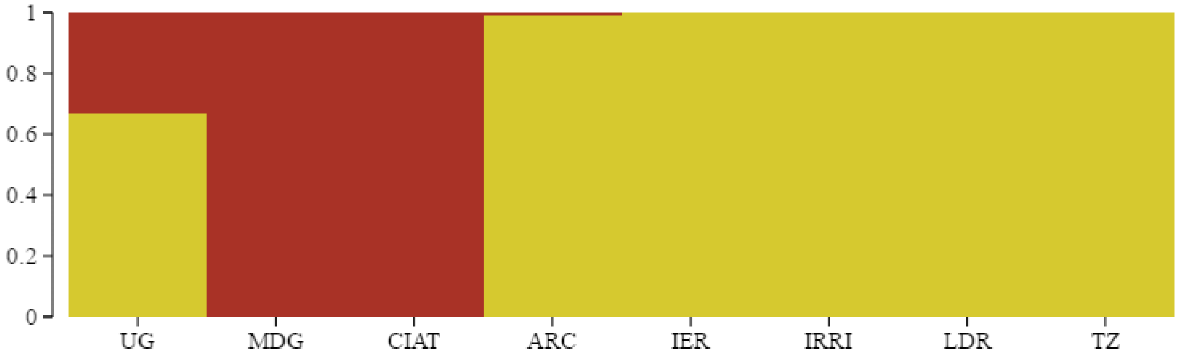


Figure 4.3: Distribution pattern of 59 rice genotypes based on Bayesian clustering method of DArTseq-derived SNP markers

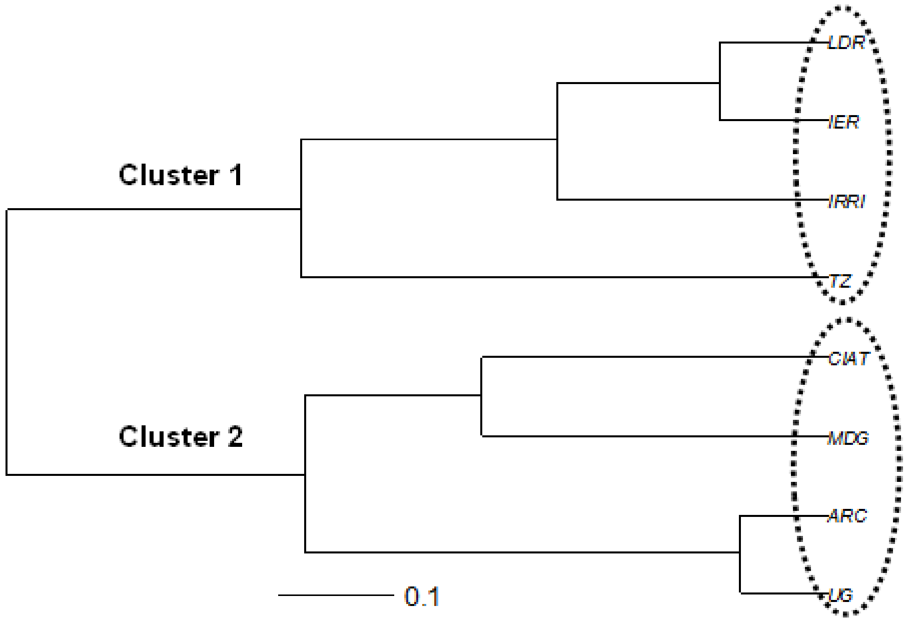


Figure 4.4: Dendrogram of a neighbour-joining (NJ) tree of rice populations constructed for 59 rice genotypes using DArTseq markers based on a mean fixation index (Fst) estimate value of 0.134

4.3.3 Principal Component analysis results

Using a 3D scatter plot of principal component analysis (PCA) and based on 525 DArTseq-derived SNPs, two major clusters were clearly distinguished among all rice populations (Figure 4.5) consistent with results from population structure analysis. Rice genotypes from cluster 1 were depicted by red colour, while cluster 2 genotypes were represented by black colour. Principal component analysis yielded three principal components accounting for 70.7% of total variance observed. Breakdown of this cumulative variance value revealed contributions of 49.5%, 15.8% and 5.4% for PCA1, PCA2 and PCA3, respectively. PCA1 was more discriminating and produced two distinct clusters.

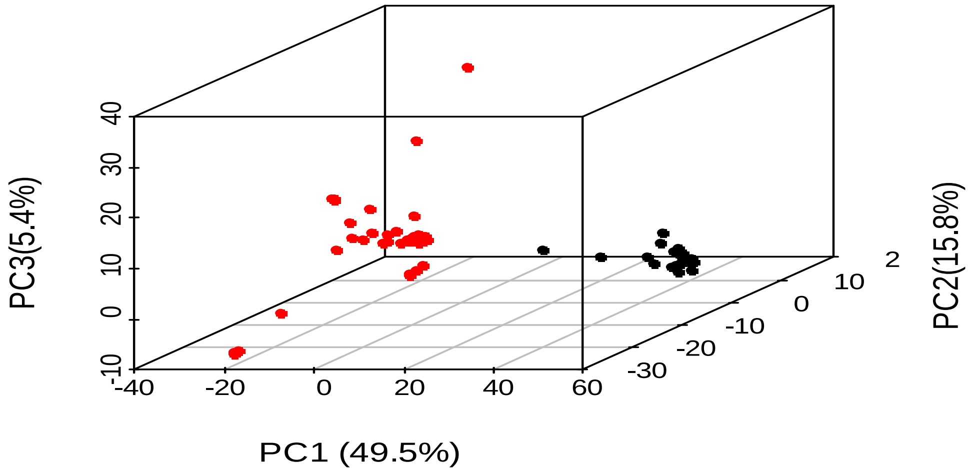


Figure 4.5: 3D scatter plot of principal component analysis for 59 rice genotypes based on DArTseq-derived SNP markers.

4.3.4 Genetic distance among populations

The genetic distance among the different populations was estimated with 525 DArTseq-derived SNP markers (Table 4.3). The greatest genetic distance was observed between genotypes from TZ and CIAT populations (0.865) and between genotypes from TZ and MDG populations (0.808). In addition, the least genetic distance was observed between genotypes from LDR and IER populations (0.004) and between genotypes from LDR and IRRI populations (0.017).

Table 4.3: Genetic distances between different populations

Populations	ARC	CIAT	IER	IRRI	LDR	MDG	TZ
CIAT	0.067	0	-	-	-	-	-
IER	0.213	0.481	0	-	-	-	-
IRRI	0.157	0.370	0.028	0	-	-	-
LDR	0.237	0.526	**0.004**	**0.017**	0	-	-
MDG	0.157	0.686	0.494	0.463	0.582	0	-
TZ	0.377	**0.865**	0.082	0.112	0.049	**0.808**	0
UG	0.023	0.228	0.218	0.194	0.267	0.069	0.399

ARC, AfricaRice; CIAT, International Centre for Tropical Agriculture; IER, Institut d'Economie Rurale – Mali; IRRI, International Rice Research Institute; LDR, Landrace-South Sudan; MDG, Madagascar; TZ, Tanzania; UG, National Crops Resources Research Institute-Uganda (NaCRRI);

4.3.5 Genetic differentiation

Analysis of molecular variance (AMOVA) among the 59 rice genotypes indicated that 11.24% of the variance was due to genetic differentiation among the populations, 67.30% of the variance was accounted by genetic differentiation among individuals within populations, while the remaining 21.46% of the variance was due to the differences within individuals (Table 4.4).

Table 4.4: AMOVA of a panel of 59 rice genotypes

Source of variation	d.f	Sum of squares	Variance components	Percentage variation
Among populations	7	2690.60	14.80	11.24
Among individuals within populations	51	10485.42	88.66	67.30
Within individuals	59	1668.00	28.27	21.46
Total	117	14844.02	131.74	

4.3.6 Phenotypic distribution of grain quality traits

Grain shape (measured as the grain length-to-width ratio) and starch related qualities such as amylose content and gelatinization temperature (measured indirectly as alkali spreading value (ASV)), are the main properties considered for selecting breeding lines with improved quality (Anacleto et al., 2015). In this study phenotypic distribution for the aforementioned grain quality traits were determined among 59 rice genotypes. Analysis of the frequency distributions of the phenotypic classes suggested that all grain quality traits were quantitative and continuous (Figure 4.6). The analysed phenotypic traits displayed an overall broad variability, which is ideal to be efficiently exploited in GWAS studies. All phenotypic traits were approximately normally distributed (Figure 4.6); a few distributions, though, were found to be slightly skewed (amylose content, grain width and length to width ratio), but none showed a clear separation in two or more classes. Grain length varied from 5.0-7.95 mm, where most of the genotypes were characterized as long grains. Grain shape ranged from 2.0-7.0 and majority of the genotypes were categorized as slender grains. The ASV varied from 1.0-6.99 which relates to high-low gelatinization temperature (GT) and most of the genotypes were grouped as intermediate GT. Percent AC ranged from 15 to 40% where majority of the genotypes were classified as intermediate AC.

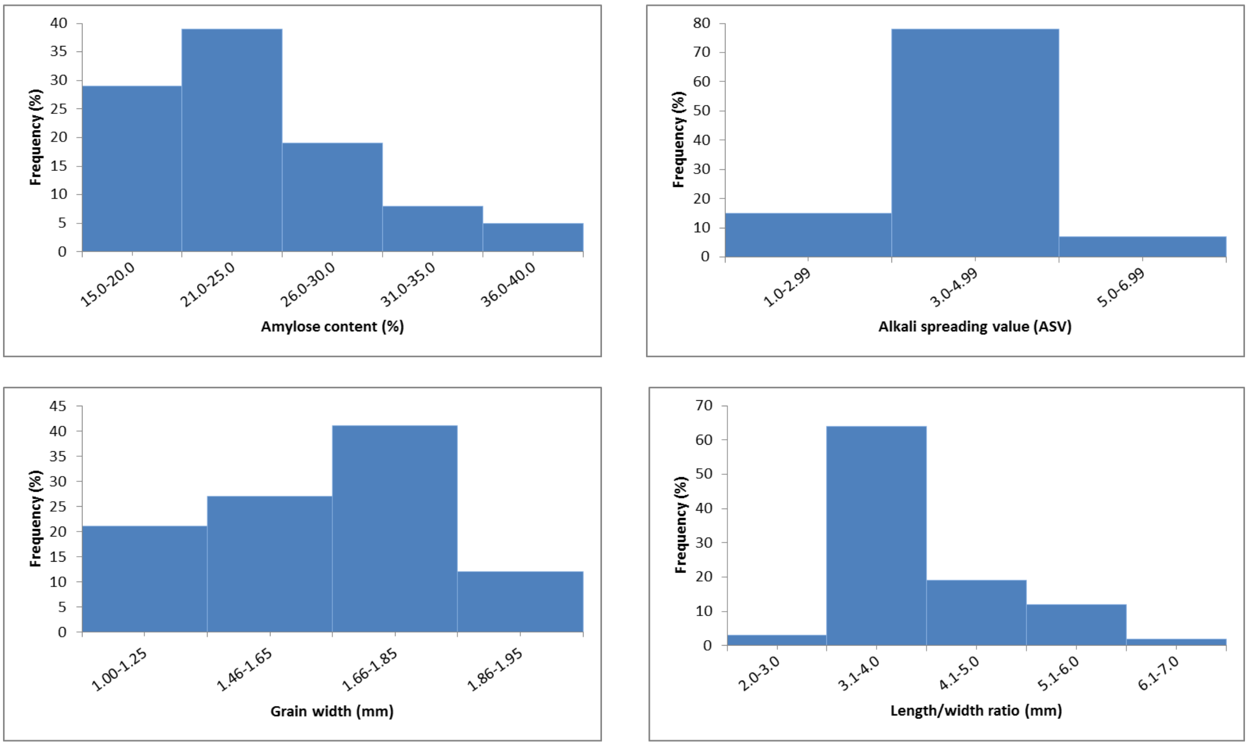


Figure 4.6: Phenotypic distribution of GWAS results for grain quality traits (AC, amylose content; ASV, alkali spreading value; GW, grain width; L/W, Grain length to width ratio); Grain shape (length/width ratio): slender≥3.0; Medium=2.1-3.0; Bold=1.1-2.0; Round<1.1; Grain length: Extra-long(≥7.5 mm); Long (6.6-7.5 mm); Medium (5.51-6.6 mm); Short (<5.51mm)

4.3.7 Genome-wide association scans for grain quality traits

Determinants of grain quality including grain length (GL), grain width (GW), grain length/width ratio (L/W), amylose content (AC) and gelatinization temperature (GT) were considered for genome-wide association studies (GWAS) using 525 DArTseq-derived SNP markers. Association mapping analysis was performed with TASSEL V5.3.1 software (Bradbury et al., 2007) using both the General Linear Model (GLM) and Mixed Linear Model (MLM) methods. Both known associations (for GW, L/W, AC and ASV) as well as candidate loci were identified, where *P*-values were used to determine the association of QTLs with markers while percent variance explained (PVE) predicted the magnitude of QTL effects. Manhattan plots for grain quality traits were generated in GWAS indicating the most significant associations (−log (*p*-value)>3) (Figure 4.7). A quantile-quantile (Q-Q) plot confirmed a normal distribution of phenotypic traits while the pattern of linkage disequilibrium (LD) blocks suggested the extent of association mapping, where the red sites represented SNPs that are in high linkage disequilibrium with each other and thus inherited together (Figure 4.8). A total of 22 significant ($P < 0.001$) association signals were detected for grain quality traits (Table 4.5). For AC, one QTL was identified on chromosome 2 that explained 48% of phenotypic variation. Ten QTLs were identified for ASV on chromosomes 1, 3, 4, 6, 7, 8, 9 and 10, contributing 19-31% of phenotypic variance. Six QTLs were also detected for GW on chromosomes 3, 5 and 12, which individually explained 23-43% of phenotypic variance. Furthermore, five QTLs were identified for L/W on chromosomes 3, 7 and 11 contributing 20-35% of phenotypic variance. SNP12_100006178, SNP13_3052560 and SNP14_3057360 (highlighted in bold) individually co-localised with two functional gene groups that are associated with QTLs for GW and L/W on chromosome 3 (Table 4.5). The AC allele (C/T) was traced back to parent K5; ASV alleles (G/A, A/G) were located in parents ART2-4L3P1-2-1, BG400-1, JARIBU and SUPA TZ; while the co-localised QTLs for GW and L/W came from JARIBU, BR4 and ART3-8L6P3-2-2-B. In general, two of the 22 associations identified were in regions where the QTL associated with the given traits had been reported in previous studies (http://www.gramene.org/ (Table 4.6)), while the remaining 20 significant SNP loci are potential novel QTLs.

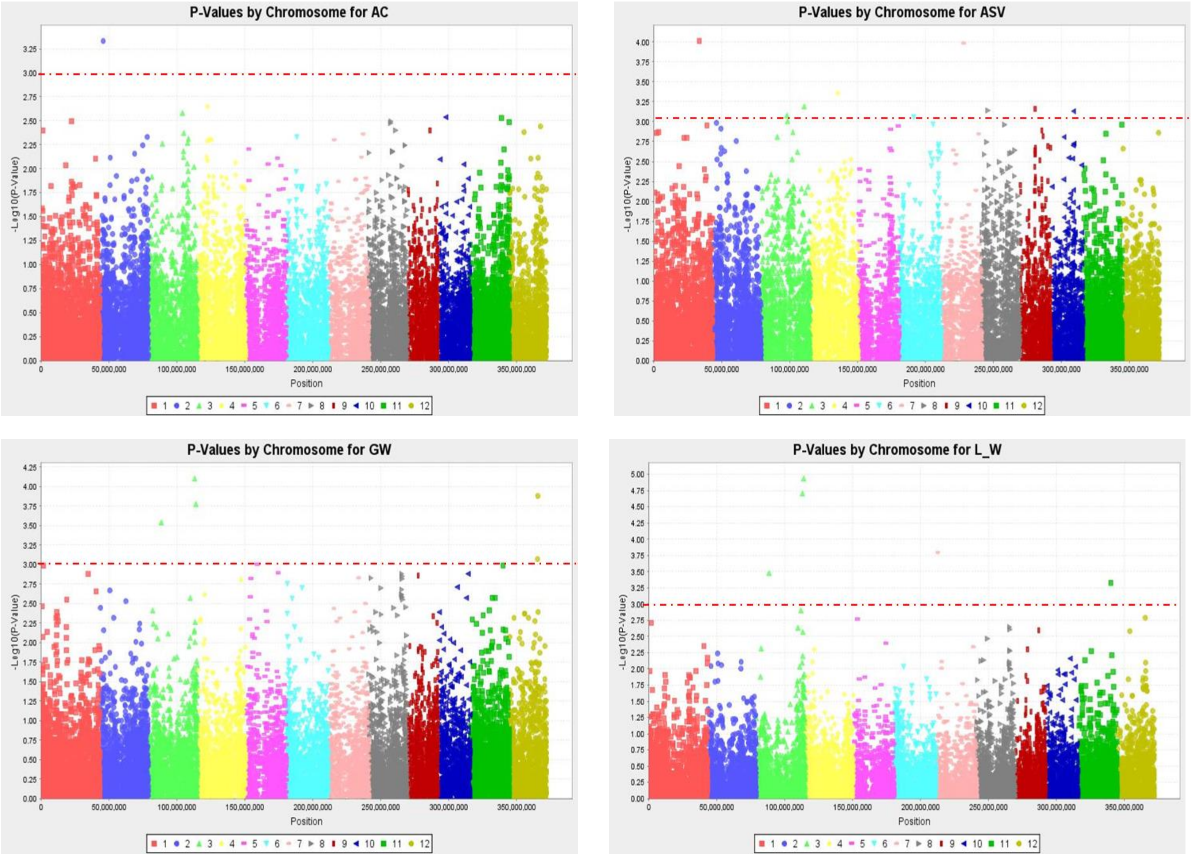


Figure 4.7: Manhattan plots of GWAS results for grain quality traits (AC, amylose content; ASV, alkali spreading value; GW, grain width; L_W, grain length to width ratio); Threshold=$-\log_{10}(p-\text{value}) > 3$

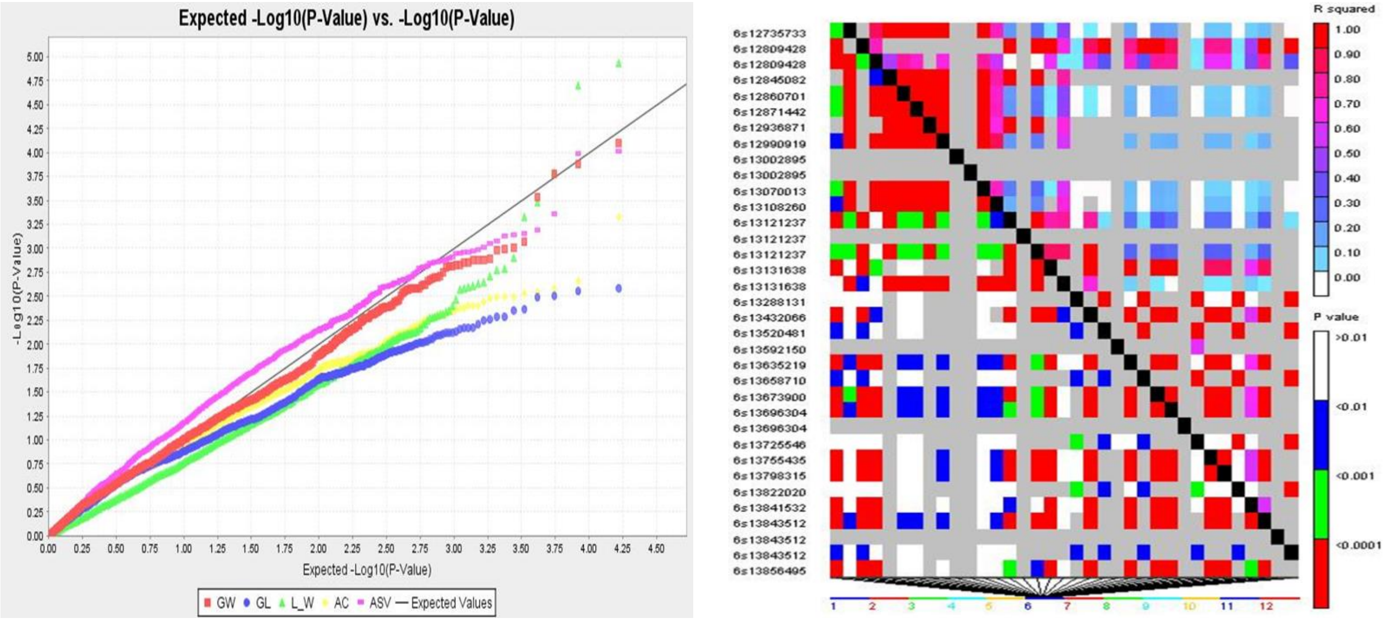


Figure 4.8: Q-Q plot (left) and patterns of LD blocks (right) of GWAS results indicating the position of candidate genes and/or QTL regions associated with grain quality traits

Table 4.5: Genome wide significant associations (R^2) of single nucleotide polymorphisms (SNPs) with amylose content (AC), alkali spreading value (ASV), grain width (GW) and grain length to width ratio (L/W)

S/No.	Grain quality trait	Marker	Chromosome	Position (cM)	ł Allele	*p*-value	PVE
1	AC	SNP1_3444982	2	16.10	C/T	4.68E-04	0.48
2	ASV	SNP2_5143094	1	144.73	G/A	9.77E-05	0.27
3	ASV	SNP3_3438578	3	163.67	A/G	6.45E-04	0.24
4	ASV	SNP4_5142766	3	106.19	G/A	8.34E-04	0.31
5	ASV	SNP5_3453263	3	106.29	A/G	9.77E-04	0.26
6	ASV	SNP6_3053581	4	45.43	A/G	4.37E-04	0.27
7	ASV	SNP7_3755646	6	55.72	C/T	8.81E-04	0.19
8	ASV	SNP8_9752845	7	80.62	G/A	1.03E-04	0.27
9	ASV	SNP9_3049990	8	34.13	G/A	7.19E-04	0.21
10	ASV	SNP10_13890040	9	25.24	C/G	6.93E-04	0.25
11	ASV	SNP11_3053914	10	49.59	G/A	7.33E-04	0.24
12	**GW**	**SNP12_100006178**	**3**	**179.45**	**C/T**	**7.93E-05**	**0.32**
13	**GW**	**SNP13_3052560**	**3**	**184.08**	**A/C**	**1.67E-04**	**0.28**
14	**GW**	**SNP14_3057360**	**3**	**55.35**	**C/T**	**2.90E-04**	**0.23**
15	GW	SNP15_3049175	5	52.50	T/A	9.82E-04	0.24
16	GW	SNP16_100003971	12	64.57	C/T	1.33E-04	0.43
17	GW	SNP17_3448915	12	63.67	A/C	8.55E-04	0.37
18	**L/W**	**SNP13_3052560**	**3**	**184.08**	**A/C**	**1.17E-05**	**0.32**
19	**L/W**	**SNP12_100006178**	**3**	**179.45**	**C/T**	**1.99E-05**	**0.35**
20	**L/W**	**SNP14_3057360**	**3**	**55.35**	**C/T**	**3.33E-04**	**0.20**
21	L/W	SNP18_100004705	7	9.27	C/T	1.60E-04	0.23
22	L/W	SNP19_9755868	11	80.06	C/A	4.67E-04	0.20

ł Allele corresponding to grain quality trait based on 59 rice genotypes ; PVE, percent variance explained

Table 4.6: Two of the 22 associations previously identified for grain quality traits

S/N0.	species	Trait name	Trait synonyms	Linkage group	Trait symbol	Published symbol	Qtl accession id	Start position (cM)	Stop position (cM)	Reference
1	*Oryza sativa* L.	grain width	KW, kernel width, width of cooked rice, width of milled rice	5	GRWD	-	CQAL27	30.2	66	Tan et al. (2000)
2	*Oryza sativa* L.	length to width ratio	LWR, grain shape, length:width ratio of the rice grain	3	GRLGW DRO	lwr3.1	AQFA014	31.2	76.9	Li et al. (2004b)

Source: http://www.gramene.org/

4.4 Discussion

Genome level profiling of rice germplasm collections is a critical initial step in identification of divergent parents for effective utilization in rice breeding programmes. The present study is the first major effort to perform genetic diversity studies and population structure analysis on a panel of 59 rice germplasm collections in South Sudan for effective breeding.

The study revealed the potential of highly informative and selective DArTseq-derived SNP markers for genetic diversity analysis and genome wide association studies in rice. Results of the diversity analysis based on geographical origin indicated that rice collections of ARC population had the highest polymorphic information content and number of alleles similar to UG population. The values were intermediate for IRRI, IER, LDR, MDG and low for TZ and CIAT populations. These results suggest that most of the rice genotypes in South Sudan are largely adopted from West Africa where the AfricaRice (ARC) gene bank is entrusted with collection, conservation and utilization of most African rice genetic resources (Sanni et al., 2013). Hence, a large number of the rice germplasm from ARC has spread to other countries within Africa such as Uganda (UG), Mali (IER), Madagascar and South Sudan. A few of the rice genotypes including accessions from IRRI and CIAT originated mainly from Asia and Latin America respectively as depicted by their geographical location.

Results of population structure analysis revealed only two major clusters and indicated a clear genetic divergence based on origin and breeding history of the rice genotypes, confirming results from principal component analysis. Genotypes were grouped into two distinct clusters based on environmental adaptation, pedigree information and genetic distances. A low mean fixation index (F_{st}) estimate value of 0.134 and a small percentage variation (11.2%) among populations as revealed by analysis of molecular variance suggested a low degree of differentiation among populations and increased levels of admixtures. Low F_{st} estimate values ranging between 0.047-0.192 were reported by Oloka et al. (2015) for rice populations sampled from IRRI, AfricaRice and NaCRRI-Uganda, and by Ogunbayo et al. (2005) for genotypes originating from AfricaRice. Semon et al. (2005) and Wang et al. (2014) suggested that the domestication of African rice may have been influenced by the introduction of Asian rice into West Africa and subsequent intercrossing. In particular, the rice population from Uganda indicated a high level of admixtures due to the on-going breeding activities. Oloka et al. (2015) reported similar findings on rice diversity studies in Uganda. Thus, based on the genetic distances between different populations, genotypes were clustered according to genetic relatedness where one cluster comprised accessions from CIAT, ARC, MDG and UG, while the other consisted of genotypes from IRRI, IER, LDR and TZ.

Analysis of frequency distributions of phenotypic classes indicated that all the grain quality traits in this study were quantitative and continuous which is in agreement with other previous studies (Lang and Buu, 2004; Lu et al., 2013; Dai et al., 2016). In addition, most of the genotypes were categorized as long and slender grains, with intermediate gelatinization temperature and amylose content. Consequently, based on the desirable characteristics of the aforementioned genotypes, they may be considered potential high market value rice grains with improved eating and cooking properties (Muhammad, 2009).

The present study also identified twenty-two significant associations with PVE of between 19-48% for rice grain quality traits in the entire set of genotypes, including 1 association with AC, 10 associations with ASV, 6 associations with GW and 5 associations with L/W. Several of the significant SNP loci were located on chromosome 3, which had previously been identified as a rice grain shape QTL hotspot region (Hu et al., 2013). Two of the 22 significant associations were in chromosomal regions in which rice grain shape QTLs had previously been located (http://www. gramene.org/). The other 20 significant SNP loci suggest the likelihood discovery of novel alleles associated with rice grain quality traits. Furthermore, SNP12_100006178, SNP13_3052560 and SNP14_3057360 individually co-localised with two functional gene groups that are associated with QTLs for grain width and grain length to width ratio on chromosome 3, indicating trait dependency or pleiotropic-effect loci. Hu et al. (2013) identified six chromosomal regions on chromosomes 1, 2, 3, 5 and 6 that had pleiotropic effects on two or more determinants of rice grain shape. Biscarini et al. (2016) also identified several significant associations that co-localised with QTLs and candidate genes controlling the phenotypic variation of single or multiple rice grain quality traits. These findings pave the way to successfully exploit genetic hot-spot regions overlapping for multiple traits to enhance predictability of superior lines in a rice breeding population. Furthermore, these results might increase the descriptive power of QTLs associated with grain quality traits in rice and thus provide useful information for further fine mapping and cloning.

4.5 Conclusion

The present study demonstrated the potential of highly informative and selective DArTseq-derived SNP markers for genetic diversity analysis and genome wide association studies in the tested rice genotypes. The study also provided a direction for breeding efforts in the selection of parents from the current collection with potential for novel genes or QTLs for important agronomic traits. A low degree of differentiation among sampled populations suggested the need for widening of the genetic base through the introduction of distant or wild relatives. Despite this, the study indicated that wide variability exists in the current rice germplasm collections for grain quality traits probably due to intercrossing between populations. Genome-wide association studies successfully identified and tagged 22 DArTseq-derived SNP loci significantly associated with rice grain quality traits. Among these, two SNP loci were found in regions where the QTL associated with the given traits had been identified in previous studies, while the remaining 20 significant associations were indicative of the likelihood discovery of novel alleles associated with rice grain quality traits. Significant QTL associations for AC allele (C/T) was traced back to parent ‘K5’; ASV alleles (G/A, A/G) were located in parents ‘ART2-4L3P1-2-1’, ‘BG400-1’, ‘JARIBU’ and ‘SUPA TZ’; while the co-localised QTLs for GW and L/W came from ‘JARIBU’, ‘BR4’ and ‘ART3-8L6P3-2-2-B’. These parents are potential sources of major effect QTLs for grain quality that can be exploited for rice crop improvement. In addition the results of this study suggest that genetic progress can be attained by intercrossing genotypes from TZ with MDG and CIAT which appear to be distantly related. Furthermore, the study identified useful targets for QTL validation; fine mapping and cloning that will help rice breeders in contributing to enhancement of rice grain quality traits through marker-assisted breeding.

References

Anacleto, R., R.P. Cuevas, R. Jimenez, C. Llorente, E. Nissila, R. Henry and N. Sreenivasulu. 2015. Prospects of breeding high-quality rice using post-genomic tools. Theoretical and Applied Genetics. 128(8): 1449-1466.

Asghar, S., F.M. Anjum, R.M. Amir and M.A. Khan. 2012. Cooking and eating characteristics or rice (*Oryza sativa* L.). Pakistan Journal of Food Sciences. 22(3): 128-132.

Bai, X., L. Luo, W. Yan, M. Kovi and W. Zhan. 2010. Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus qGL7. BMC Genetics. 11(1): 16.

Biscarini, F., P. Cozzi, L. Casella, P. Riccardi, A. Vattari, G. Orasen, R. Perrini, G. Tacconi, et al. 2016. Genome-wide association study for traits related to plant and grain morphology, and root architecture in temperate rice accessions. Plos One 11: e0155425.

Biselli, C., D. Cavalluzzo, R. Perrini, A. Gianinetti, P. Bagnaresi, S. Urso, G. Orasen, F. Desiderio, et al. 2014. Improvement of marker-based predictability of Apparent Amylose Content in japonica rice through GBSSI allele mining. Rice. 7(1): 1.

Boutet, G., S.A. Carvalho, M. Falque, P. Peterlongo, E. Lhuillier, O. Bouchez, C. Lavaud, M.L. Pilet-Nayel, et al. 2016. SNP discovery and genetic mapping using Genotyping-by-sequencing of whole genome genomic DNA from a pea RIL population. BMC Genomics. 17(1): 1.

Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss and E.S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 23(19): 2633-2635.

Brar, D. and G. Khush. 1997. Alien gene introgression in rice. Plant Molecular Biology 35(1): 35-47.

Courtois, B., A. Audebert, A. Dardou, S. Roques, T. Ghneim-Herrera, G. Droc, J. Frouin, L. Rouan, et al. 2013. Genome-wide association mapping of root traits in a japonica rice panel. Plos One 8: e78037.

Cruz, N.D. and G. Khush. 2000. Rice grain quality evaluation procedures. Aromatic Rices 3: 15-28.

Dai, L., L. Wang, Y. Leng, Y. Yang, L. Huang, L. Chen, Y. Wang, D. Ren, et al. 2016. Quantitative Trait Loci Mapping for Appearance Quality in Short-Grain Rice. Crop Science 56: 1484-1492.

Das, B., S. Sengupta, S. Parida, B. Roy, M. Ghosh, M. Prasad and T. Ghose. 2013. Genetic diversity and population structure of rice landraces from Eastern and North Eastern States of India. BMC Genetics. 14(1): 71.

Demont, M. 2013. Reversing urban bias in African rice markets: A review of 19 National Rice Development Strategies. Global Food Security. 2(3): 172-181.

Dobo, M., N. Ayres, G. Walker and W.D. Park. 2010. Polymorphism in the GBSS gene affects amylose content in US and European rice germplasm. Journal of Cereal Science 52: 450-456.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. Plos One 6: e19379.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14(8): 2611-2620.

Furuta, T., M. Ashikari, K.K. Jena, K. Doi and S. Reuscher. 2017. Adapting Genotyping-by-Sequencing for Rice F2 Populations. G3: Genes| Genomes| Genetics: g3. 116.038190.

Garris, A., T. Tai, J. Coburn, S. Kresovich and S. McCouch. 2005. Genetic structure and diversity in *Oryza sativa* L. Genetics 169: 1631-1638.

Gibson, T., V. Solah and B. McCleary. 1997. A procedure to measure amylose in cereal starches and flours with concanavalin A. Journal of Cereal Science 25: 111-119.

Gupta, P., S. Rustgi and R. Mir. 2008. Array-based high-throughput DNA markers for crop improvement. Heredity 101: 5-18.

He, J., X. Zhao, A. Laroche, Z.-X. Lu, H. Liu and Z. Li. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in Plant Science. 5: 484.

Hu, W., M. Wen, Z. Han, C. Tan and Y. Xing. 2013. Scanning QTLs for grain shape using a whole genome SNP array in rice. Journal of Plant Biochemistry and Physiology. 1(1): 104.

Huang, B.E., A.W. George, K.L. Forrest, A. Kilian, M.J. Hayden, M.K. Morell and C.R. Cavanagh. 2012. A multiparent advanced generation inter-cross population for genetic analysis in wheat. Plant Biotechnology Journal. 10(7): 826-839.

Jarquín, D., K. Kocak, L. Posadas, K. Hyma, J. Jedlicka, G. Graef and A. Lorenz. 2014. Genotyping by sequencing for genomic prediction in a soybean breeding population. BMC Genomics. 15(1): 740.

Jiang, G., X. Hong, C. Xu, X. Li and Y. He. 2005. Identification of quantitative trait loci for grain appearance and milling quality using a doubled-haploid rice population. Journal of Integrative Plant Biology 47: 1391-1403.

Juliano, B.O. 1985. Rice: chemistry and technology. American Association of Cereal Chemists St Paul, MN.

Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia, H. Blois, V. Caig, K. Heller-Uszynska, et al. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. Data Production and Analysis in Population Genomics: Methods and Protocols: 67-89.

Koutroubas, S.D., F. Mazzini, B. Pons and D.A. Ntanos. 2004. Grain quality variation and relationships with morpho-physiological traits in rice (*Oryza sativa* L.) genetic resources in Europe. Field Crops Research 86: 115-130.

Lang, N.T. and B.C. Buu. 2004. Quantitative analysis on amylose content by DNA markers through backcross populations of rice (*Oryza sativa* L.). OMonRice 12: 12-17.

Li, J., M. Thomson and S. McCouch. 2004a. Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. Genetics 168: 2187-2195.

Li, J., J. Xiao, S. Grandillo, L. Jiang, Y. Wan, Q. Deng, L. Yuan and S.R. McCouch. 2004b. QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome 47(4): 697-704.

Little, R.R., G.B. Hilder and E.H. Dawson. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry. 35(2): 111-126. .

Lu, B.Y., C.Y. Yang, K. Xie, L. Zhang, T. Wu, L.F. Li, X. Liu, L. Jiang, et al. 2013. Quantitative trait loci for grain-quality traits across a rice F-2 population and backcross inbred lines. Euphytica 192: 25-35.

Malathi, S., B. Divya, M. Sukumar, K. Addanki, V. Yadavalli and N. Sarla. 2016. Genetic characterization and population structure of Indian rice cultivars and wild genotypes using core set markers. 3 Biotech 6(1): 95.

Muhammad, A. 2009. Aromatic Rices of Pakistan-a review. Pakistan Journal of Agricultural Research 22: 154-160.

Ogunbayo, S., D. Ojo, R. Guei, O. Oyelakin and K.I. Sanni. 2005. Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques. African Journal of Biotechnology 4(11): 1234-1244.

Oloka, B.M., J. Lamo, P. Rubaihayo, P. Gibson and J. Vorster. 2015. The use of multiplexed simple sequence repeat (SSR) markers for analysis of genetic diversity in African rice genotypes. African Journal of Biotechnology 14: 1533-1542.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Resources 6: 288-295.

Poland, J.A. and T.W. Rife. 2012. Genotyping-by-sequencing for plant breeding and genetics. The Plant Genome 5: 92-102.

Pritchard, J.K., M. Stephens, N.A. Rosenberg and P. Donnelly. 2000. Association mapping in structured populations. The American Journal of Human Genetics 67: 170-181.

Pritchard, J.K., W. Wen and D. Falush. 2003. Documentation for structure software: version 2.

Qiu, X., R. Gong, Y. Tan and S. Yu. 2012. Mapping and characterization of the major quantitative trait locus qSS7 associated with increased length and decreased width of rice seeds. Theoretical and Applied Genetics 125: 1717-1726.

Ramasamy, R.K., S. Ramasamy, B.B. Bindroo and V.G. Naik. 2014. STRUCTURE PLOT: a programme for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3: 431.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4): 406-425.

Sánchez-Sevilla, J.F., A. Horvath, M.A. Botella, A. Gaston, K. Folta, A. Kilian, B. Denoyes and I. Amaya. 2015. Diversity Arrays Technology (DArT) marker platforms for diversity analysis and linkage mapping in a complex crop, the octoploid cultivated strawberry (Fragaria× ananassa). Plos One 10: e0144960.

Sanni, K.A., D.D. Tia, D.K. Ojo, A.S. Ogunbayo, M. Sikirou and N.R.S. Hamilton. 2013. Diversity of rice and related wild species in Africa. Realizing Africa's rice promise. CABI, Boston: 87-94.

Semon, M., R. Nielsen, M.P. Jones and S.R. McCouch. 2005. The Population Structure of African Cultivated Rice *Oryza glaberrima* (Steud.). Genetics 169: 1639-1647.

Tadesse, W., F. Ogbonnaya, A. Jighly, M. Sanchez-Garcia, Q. Sohail, S. Rajaram and M. Baum. 2015. Genome-wide association mapping of yield and grain quality traits in winter wheat genotypes. Plos One 10: e0141339.

Tan, Y.F., Y.Z. Xing, J.X. Li, S.B. Yu, C.G. Xu and Q. Zhang. 2000. Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. Theoretical and Applied Genetics. 101(5): 823-829.

Tang, W., T. Wu, J. Ye, J. Sun, Y. Jiang, J. Yu, J. Tang, G. Chen, et al. 2016. SNP-based analysis of genetic diversity reveals important alleles associated with seed size in rice. BMC Plant Biology. 16(1): 93.

Traoré, V.S., B.J. Néya, M. Camara, V. Gracen, S.K. Offei and O. Traoré. 2015. Farmers' Perception and Impact of Rice Yellow Mottle Disease on Rice Yields in Burkina Faso. Agricultural Sciences 6: 943.

Vanaja, T. and T.B. Luckins. 2006. Variability in grain quality attributes of high yielding rice varieties (*Oryza sativa* L.) of diverse origin. Journal of Tropical Agriculture. 44(1-2): 61-63.

Von Mark, V.C., A. Kilian and D.A. Dierig. 2013. Development of DArT marker platforms and genetic diversity assessment of the US collection of the new oilseed crop *lesquerella* and related species. Plos One 8: e64062.

Wang, M., Y. Yu, G. Haberer, P.R. Marri, C. Fan, J.L. Goicoechea, A. Zuccolo, X. Song, et al. 2014. The genome sequence of African rice (*Oryza glaberrima*) and evidence for independent domestication. Nature Genetics 46: 982-988.

Wang, X.Q., L.Q. Yin, G.Z. Shen, L. Xu and Q.Q. Liu. 2010. Determination of Amylose Content and Its Relationship with RVA Profile Within Genetically Similar Cultivars of Rice (*Oryza sativa* L. *ssp. japonica*). Agricultural Sciences in China 9: 1101-1107.

Xiao, J., J. Li, S. Grandillo, S. Ahn and L. Yuan. 1998. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150: 899-909.

Yu, J., G. Pressoir, W.H. Briggs, I.V. Bi, M. Yamasaki, J.F. Doebley, M.D. McMullen, B.S. Gaut, et al. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38: 203-208.

Zhang, X., J. Wang, J. Huang, H. Lan and C. Wang. 2012. Rare allele of OsPPKL1 associated with grain length causes extra-large grain and a significant yield increase in rice. Proceedings of the National Academy of Sciences,U.S.A. 109: 21534-21539.

5. Genetic variability and quantitative trait loci analysis for yield-related and grain quality traits in three rice F_2 populations

Abstract

Grain yield and quality improvement are amongst the most important goals in any rice breeding program. However, the complex nature of these traits and their interaction with the environment lessens breeding progress. The main objective of this study was to perform quantitative trait loci (QTL) mapping for yield-related and grain quality traits of rice using three independent F_2 populations. In this respect, *Oryza sativa* L. rice genotypes (‘Basmati 370’, ‘Komboka’ and ‘Supa 1052’) with improved grain quality but low yield were crossed to an interspecific hybrid (*O. sativa* L. x *O. glaberrima* Steud) ‘NERICA 4’ with improved yield but poor grain quality. A high-density genetic linkage map was constructed via genotyping F_2 mapping populations by DArTseq-derived SNP markers. Estimates of genotypic and environmental variances, heritability and genetic advance as a percentage of mean for grain yield and quality traits among parents, F_2 plants and BC_1F_2 individuals were derived. Relatively low differences (<10.0) between the phenotypic and genotypic coefficient of variation were observed for grain length, grain width and alkali spreading value among parents and progenies of segregating populations. Moderate to high heritability values coupled with high genetic advance were observed for alkali spreading value and grain yield. A total of 23 main-effect QTLs were uncovered for grain length, grain width, amylose content and alkali spreading value. Each QTL individually explained between 0.6% and 54.8% of the total phenotypic variance. Both novel and previously reported main-effect QTLs were identified. Novel main-effect QTLs for low AC (*qAC-8-1* and *qAC-8-5)* were detected on chromosome 8 and accounted for 2.7% and 10.3% of the phenotypic variance, respectively. Further, a major QTL (*qGL-12-5)* for grain length with a positive additive effect, explaining 30.3% of phenotypic variance on chromosome 12 was identified as a potential novel QTL. The results suggest that hybridization with *O. sativa* and interspecific hybrids can be successfully exploited to improve grain yield and quality in rice. Other main-effect QTLs for yield-related and grain quality traits such as; *qAC-6-4, qGL-4-2*, *qGW-5-3* and *qASV-3-5* that individually explained between 14%-45.8% of the total phenotypic variance, were consistent with observations from other studies. Novel QTLs detected in this study for yield-related and grain quality traits could be considered as potential candidates for future fine-mapping and positional cloning projects.

Keywords: Rice, Grain yield and quality, Quantitative trait loci, DArTseq-derived SNP markers, Heritability, Genetic advance

5.1 Introduction

Rice (*Oryza sativa* L.) forms an essential component of the human diet in many regions of the world and provides an important source of calories for at least 50% of the world's population (Asante, 2017). There is a growing global demand for rice breeding programs to focus on enhancing rice yield coupled with superior grain quality (Sreenivasulu et al., 2015; Zhao et al., 2015) in order to ensure food security. This challenge is even more critical in sub-Saharan Africa (SSA) where the current growth in population, increased urbanization and changes in consumer preferences has caused a rise in the demand for high quality rice (Balasubramanian et al., 2007). However, local rice production is low and does not match imported rice in end-user qualities (Yi et al., 2009; Demont, 2013). Furthermore, given the high preference for imported rice, close to 40% of the rice consumed in SSA is imported (Seck et al., 2010). Therefore, improving rice yield potential and grain quality is important but poses a great challenge to rice breeders because of the complex nature of these traits and their interaction with the environment (Wang et al., 2012a; Yacouba et al., 2013). According to Swamy et al. (2012), consumer preference for rice is largely determined by appearance and cooking and eating qualities. In addition, grain size and shape are important components of grain yield and quality (Wang et al., 2012b) and relate to grain appearance. Grain appearance is specified by grain length (GL), width (GW), length–width ratio (L/W), colour, and translucency of polished grains. Furthermore, cooking and eating qualities are largely governed by amylose content (AC), gel consistency (GC), gelatinization temperature (GT) or alkali spreading value (ASV), and pasting properties of starch.

Yield and grain quality traits are complex and follow quantitative inheritance with considerable influence of genotype, environment, and their interactions (Wang et al., 2007; Amarawathi et al., 2008), thus, breeding for their right combinations using phenotype-based classical breeding is difficult and inefficient (Swamy et al., 2012). However, molecular marker technology provides options for improving selection strategies (Akhtar et al., 2010), and facilitates the selection of complex traits during the breeding process because they are reliable and unaffected by environmental conditions (Yi et al., 2009). Further, with the current advancements made in DNA marker technology and the development of linkage maps for rice, it is possible for complex polygenic traits to be dissected into single Mendelian quantitative trait loci (QTL) (Lou et al., 2009). Thus, several QTLs for traits of agronomic importance have been identified and used in rice improvement by marker-assisted selection (MAS) (Liu et al., 2013; Lu et al., 2013). Furthermore, mapping of QTLs for yield-related and grain quality traits in rice is an important genetic approach to dissect and integrate the traits in marker-assisted breeding and for gene discovery. In rice, several of these QTLs have been mapped and tagged in different genetic backgrounds using molecular markers (Tan et al., 1999; Moncada et al., 2001; Aluko et al., 2004; Wang et al., 2007; Wan et al., 2008; Zhou et al., 2009; Jing et al., 2010). Previous QTL mapping studies involving different mapping populations have revealed the complex nature of both yield-related and grain quality traits and suggested that several chromosomal regions are associated with the expression of a given phenotype. Aluko et al. (2004) mapped a QTL for grain length on chromosome 3, explaining 12.5% of the phenotypic variation (PV). Amarawathi et al. (2008) identified three significant QTLs for grain length, one on chromosome 1 and two on chromosome 7 accounting for 10% and 7% of PV. Lou et al. (2009) detected 3 QTLs for amylose content on chromosome 2 which collectively explained 2.55% of the PV. Yacouba et al. (2013) identified two QTLs for AC on chromosome 6 in 2009 and another QTL on chromosome 8 in 2010 that individually accounted for 8.6% and 5.7% of the total phenotypic variation respectively.

In addition, the presence of epistasis and QTL x environment interaction makes it difficult to apply MAS for genetic improvement of complex traits (Xu and Crouch, 2008). Furthermore, the formation of QTL clusters due to a single gene with pleiotropic effects or due to the ‘‘linkage drag’’ of multiple linked genes, each affecting a different trait, limits breeding efforts (Yamamoto et al., 2009). Nevertheless, efforts in QTL mapping and validation has not only improved the use of MAS for transfer of desirable QTL clusters and unlocking of undesirable linkage, but also provided insight into the genetic mechanisms regulating related traits (Wang et al., 2012a). Yi et al. (2009), successfully improved fragrance and intermediate AC in a Myanmar rice cultivar (Manawthukha) by marker-assisted backrossing (MABC). Other achievements in improving complex traits by marker-assisted selection (MAS) using advanced backcross populations, chromosomal segment substitution lines, near-isogenic lines (NIL), and heterogeneous inbred families (HIF) with uniform genetic background have been reported (Xie et al., 2008; Maas et al., 2010; Tyagi et al., 2014).

Grain yield and quality are key traits when defining the desirability of rice (Lu et al., 2013). Thus, a comprehensive understanding of the genetic basis for those traits is particularly helpful in making improvements to both grain yield and marketing quality. The main objective of the investigation reported here was to map QTLs for yield-related and grain quality attributes of rice using three F_2 populations derived from the cross between an *O. sativa* rice cultivar and interspecific O*. sativa* L. x *O. glaberrima,* Steud, hybrid. The identified QTLs will facilitate the development of strategies for improving yield-related and grain quality traits of farmer preferred cultivars by identifying new genetic resources with enhanced grain quality attributes. The study will also improve our understanding of the genes, pathways and molecular mechanisms determining yield-related and grain quality traits in rice.

5.2 Materials and methods

5.2.1 Plant material and mapping population

In the present study 3 mapping populations were derived from a cross between ‘NERICA 4’ (male parent) and ‘Basmati 370’, ‘Komboka’ and ‘Supa 1052’ (female parents). The male parent is an interspecific hybrid of a cross between *O. glaberrima* Steud and *O. sativa* L. It is a popular, high-yielding upland rice variety released in South Sudan in 2015 but is less preferred to imported rice such as ‘Basmati 370’, ‘Komboka’ and ‘Supa 1052’ in grain quality attributes. The female parents are *O. sativa* cultivars that have desirable superior grain quality traits but are low yielding and less adapted to the rice ecology in the country. Twenty F1 seeds generated for each of the 3 crosses were pre-germinated in petri-dishes. At least 120 individuals of F_2 plants and 50 backcross inbred plants (BC_1F_2) were generated for each population.

5.2.2 Experimental setup

Field evaluations were conducted at the National Crops Resources Research Institute, (NaCRRI) in Uganda. NaCRRI experiences a bimodal rainfall pattern and receives an average annual rainfall of 1200 mm. It is located at 00^0 32″ N and 32^0 37″ E of the Equator at approximately 27 km north of Kampala at an elevation of 1150 metres above sea level (m asl). It has a tropical wet and mild dry climate with slightly humid conditions (average 65%). The soil type at the experimental site was clay vertisol. The evaluations were done during the 2016/2017 second growing season (April-September) under irrigated conditions. F_2 populations, backcross inbred lines (BILs) and their parents were grown in 3 replications in a split-split-plot design arranged in RCBD. Seasons were considered as whole plots, generations as sub-plots and populations as sub-sub plots. Thus populations were nested within generations and generations within seasons. Each generation of F_2 plants, backcross inbred plants and parents consisted of 30 plants planted in 3 rows of 10 plants each adopting a uniform spacing of 25 cm between rows and 15 cm between plants. Standard cultural management practices including fertilisation and weeding were uniformly applied throughout the crop growth period. The crops were fertilized with 25 kg N ha^{-1} at 20-25 DAE and the same rate at 40-45 DAE to enhance plant vigour.

5.2.3 Data collection

5.2.3.1 Grain quality traits

Individual plants were assessed for determinants of kernel quality including kernel size-shape, amylose content, alkali spreading value and other important agronomic characters on yield performance. Kernel quality was determined using dehusked grains. Kernels were classified on the basis of length (L) and width (W) in three replicates using a vernier calliper following classification described by Cruz and Khush (2000). Amylose and amylopectin content of the starch was determined by the method of Gibson et al. (1997) using a Megazyme amylose/amylopectin assay kit (K-AMYL 04/06, Megazyme International Ireland Ltd., Co. Wicklow, Ireland), as described in the previous chapter (see chapter 3, section 3.3.3). Gelatinization temperature (GT) was assessed indirectly as the alkali spreading value of hulled kernels as per modified procedure of Little et al. (1958) as illustrated in chapter 3, section 3.3.4.

5.2.3.2 Agro-morphological traits

Agronomic traits measured included days to heading (DH), plant height (PH), number of productive tillers per plant (NETP), number of grains per panicle (NGP), panicle length (PL), percent spikelet fertility (PSF), weight of 1000 grains (TGW) and grain yield (GY). Days to heading were recorded when 50% of the plants in each plot had flowered, while PH, NETP and NGP were measured at maturity and based on ten individual plants randomly selected in each plot. PH was measured from the soil surface to the tip of the panicle, while PL was measured from the node of the panicle to the tip of the panicle. Phenotypic acceptability and proneness to lodging was recorded visually according to the rice Standard Evaluation System (SES) described by IRRI (2002). Only the inner row was considered for measurement of GY in each plot, whilst the grain moisture content was adjusted to 14% and the GY per plot extrapolated to tonnes per hectare.

5.2.3.3 DNA extraction and genotyping

Total genomic DNA was isolated from three-week old leaves of individual plants using the ZYMO research *Quick*-DNA™ Plant/Seed 96 Kit. For each of the three F_2 mapping populations, 94 individual plants were selected giving a total of 282 individual plants. Isolated DNA were checked for purity and concentration using the Thermo Scientific™ NanoDrop 2000 spectrophotometer as described in the user’s manual, while the DNA quality was verified by running aliquots of DNA samples on a 1% agarose gel that contained 0.5 µg/mL GelRed. Consequently, 40 µl of a 50 ng/µl DNA of each sample was sent to Diversity Arrays

Technology (DArT) Pty Ltd, Australia ('http://www.diversityarrays.com/dart-map-sequences') for whole genome scan using DArTseq-derived SNP markers. Whole-genome genotyping was carried out using GBS technology as described by Elshire et al. (2011). A total of 15,726 candidate DArTseq-derived SNP markers were discovered. DArTseq-derived SNP markers were filtered to remove bad SNPs and genotypes using PLINK 1.9 software in MS window and R statistical software, where genotypes with > 20% missing data, SNP loci with >20% missing data and rare SNPs with <5% minor allele frequencies (MAF) were pruned. This reduced the number of quality SNPs to 9452 (Figure 5.1) which were used to genotype 282 individual F_2 plants.

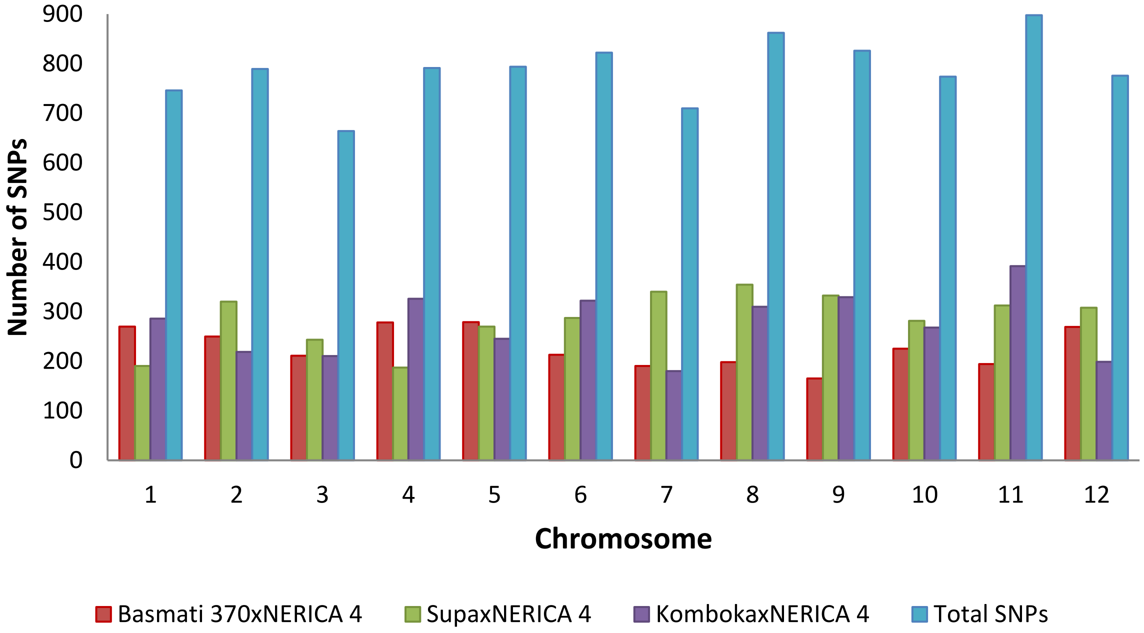


Figure 5.1: Summary of the number of SNPs used for QTL mapping for each mapping population and combined across all populations

5.2.4 Data analysis

5.2.4.1 Agronomic and grain quality traits

Data analyses were performed using GenStat 14th Edition software (VSN, 2011) using the analysis of variance (ANOVA) procedure followed by mean comparisons for agronomic characters and grain quality traits. A split-split-plot analysis with main plots arranged in RCBD was used where seasons (S) were considered as whole plots, generations (G) as sub-plots and populations (P) as sub-sub plots. The linear model for split-split-plot analysis was as follows;

$Y_{ijkl} = \mu_{ijk} + Si + R(S)_{ij} + PK + SP_{ik} + PR(S)_{ijk} + G_l + GS_{il} + GP_{kl} + GPS_{ikl} + E_{ijkl}$

Where, μ_{ijk} denotes the mean for level $_i$ of seasons (S), level $_{j}$ of populations (P) and level $_k$ of generations (G).

Mean separation was conducted using the least significance difference (LSD) at 5% probability. Frequency distributions of grain quality trait means for the 3 mapping populations were generated by Excel 2010.

5.2.4.2 Mean performance and heritability estimates for grain yield and quality traits

Genotypic and environmental variances were estimated for parents, F_2 populations and BC_1F_2 individuals using the formulae for expected variances in the model, while phenotypic variance was estimated as the sum of genotypic and environmental variances. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated following the formulae by Burton (1952). Broad sense heritability [H^2] was estimated as described by Lush (1949) and Johnson et al. (1955) according to the following equations;

Phenotypic coefficient of variation = $\frac{\sqrt{\sigma^{2p}}}{\bar{x}}$ x100

Genotypic coefficient of variation = $\frac{\sqrt{\sigma^{2g}}}{\bar{x}}$ x100

Broad sense heritability [H^2] = $\frac{\sigma^{2g}}{\sigma^{2p}}$x100

Where;

$\bar{x}$ = Mean of the characteristic

σ^{2g} = Genotypic variance

σ^{2p} = Phenotypic variance

Genetic advance (GA) as a percent of the mean was estimated by the formula suggested by Johnson et al. (1955) as follows;

Genetic advance (% of mean) = $\frac{K\sigma p h^2}{\bar{x}}\ x\ 100$

where,

σp = Phenotypic standard deviation

K = Selection differential [2.06 at 5 % selection intensity (Allard, 1960)].

H^2 = Broad sense heritability

$\bar{x}$ = Mean of characteristic

Broad sense heritability estimates were classified as low (< 30 %), medium (30 – 60 %) and high (> 60 %), while genetic advance was categorised as low (< 10 %), moderate (10 – 20 %) and high (> 20 %) as described by Johnson et al. (1955).

5.2.4.3 Linkage map construction and QTL analysis

A linkage map was constructed from the mapping population genotyping data using the integrated breeding platform (IBP) and breeding management system (BMS) software tool (IBP, 2015). For map distance calculations, recombination frequencies were converted to centiMorgans (cM) using Kosambi's method (Kosambi, 1944). DArTseq-derived SNP markers were integrated into a linkage map by inferring marker order and position from the consensus DArT map. QTLs were estimated by inclusive composite interval mapping (ICIM) using the integrated breeding platform (IBP) and breeding management system (BMS) software tool (IBP, 2015). The relative contribution of a genetic component was calculated as the proportion of the additive effect and phenotypic variance explained by that component. Logarithm of odds (LOD) thresholds for QTL significance were determined by a permutation test (1000 replications) with a genome-wide significance level P=0.01 to judge whether there exist QTLs. Interval mapping at 1-cM intervals along the chromosomes was then used to scan for QTLs. Markers closely linked to positions with the highest LOD score were taken as cofactors for ICIM analysis. To select significant markers during the first step of ICIM stepwise regression, *P*-values for entering and removing variables were set at 0.001 and 0.002 respectively; in the second step, a minimum LOD threshold of 3.0 was used to declare a QTL significant. QTL nomenclature followed the protocol of Mccouch et al. (1997). For instance, "*qAC-8-1*" denotes the first QTL associated with AC expression and is detected and located on the eighth linkage group.

5.3 Results

5.3.1 Phenotypic variance for grain quality traits in F_2 mapping populations

The three F_2 mapping populations showed a continuous and normal distribution for grain quality traits (GL, GW, AC and ASV) and also indicated transgressive segregations (Figure 5.2). Notable differences were observed between the measured traits from respective parents involved in crosses and among the F_2 mapping populations (Figure 5.2). Trait differences between parents involved in crosses and F_2 mapping populations provided a rich source of trait variation for population development and QTL mapping.

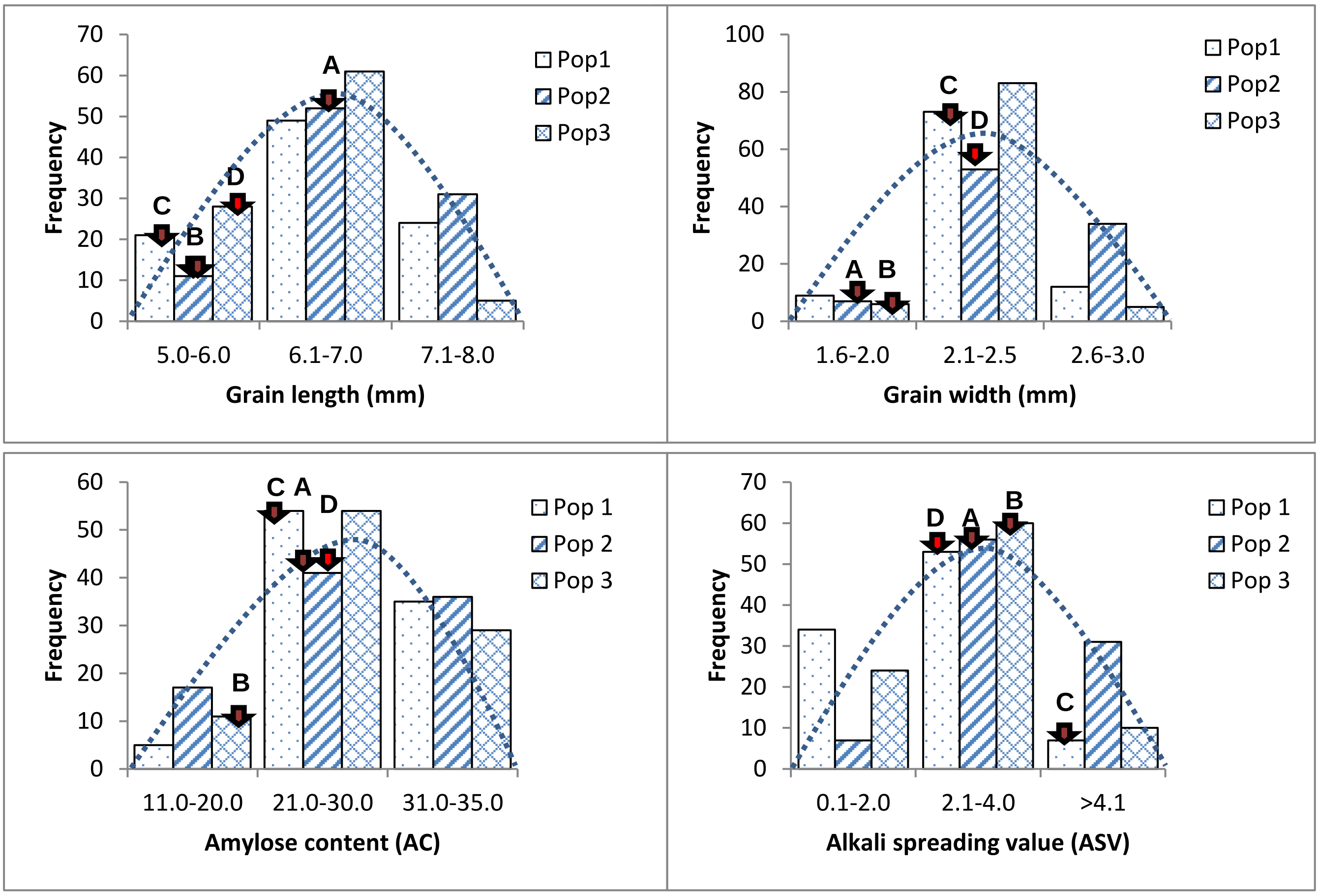


Figure 5.2: Frequency distributions of grain length, grain width, amylose content and alkali spreading value in 3 F_2 mapping populations showing a good fit to the normal distribution; Pop 1, 'Basmati 370 x NERICA 4'; Pop 2, 'Komboka x NERICA 4'; Pop 3, 'Supa 1052 x 'NERICA 4'; Parent A, 'Basmati 370'; Parent B, 'Komboka'; Parent C, 'Supa 1052'; Parent D, 'NERICA 4'.

5.3.2 Significant tests and heritability estimates for grain yield and quality trait

Summary results on tests for significance, phenotypic and genotypic variation and heritability estimates for grain yield and quality traits among 3 independent populations is as follows;

'*Basmati 370 x NERICA 4' populations*

Analyses of variance for generations of parents, F_2 plants and BC_1F_2 individuals consistently demonstrated highly significant differences ($p<0.01$ and $p<0.001$) for grain yield and quality traits (Table 5.1). Significant differences ($p<0.05$) were also observed for the interaction between seasons and generations (Table 5.1). Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits investigated in this study, where the highest PCV and GCV values were recorded for GY and ASV, while the lowest values were recorded for AC, GW and GL (Table 5.2). The difference between genotypic and phenotypic coefficient of variation was small (<10.0) for all characters studied except for GY and AC (Table 5.2). Broad sense heritability estimates (H^2) for grain yield and quality traits ranged from low to intermediate and high, but varied considerably from trait to trait (Table 5.2). The highest heritability was recorded for ASV, while AC indicated the lowest heritability estimate (Table 5.2). Genetic advance as a percent of mean was highest for ASV followed by GY, while the lowest was recorded for AC (Table 5.2).

'Komboka x NERICA 4' populations

Generations of parents, F_2 plants and BC_1F_2 individuals consistently recorded highly significant differences ($p<0.001$) for grain yield and quality traits (Table 5.1). Significant differences ($p<0.05$) were also noted for the interaction between seasons and generations (Table 5.2). Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits investigated, where the highest PCV and GCV values were recorded for GY and ASV, while the lowest values were recorded for AC, GW and GL (Table 5.2). The difference between genotypic and phenotypic coefficient of variation was small (<10.0) for all characters studied except for GY (Table 5.2). Broad sense heritability estimates (H^2) for grain yield and quality traits ranged from intermediate to high, and were variable from trait to trait (Table 5.2). The highest heritability was recorded for ASV, while GL indicated the lowest heritability estimate (Table 5.2). Genetic advance as a percent of mean was highest for GY followed by ASV, while the lowest was recorded for GL (Table 5.2).

'Supa 1052 x NERICA 4' populations

Highly significant differences ($p<0.001$) were recorded for grain yield and quality traits among generations of parents, F_2 plants and BC_1F_2 individuals (Table 5.1). Significant differences ($p<0.01$) were also noted for the interaction between seasons and generations (Table 5.1). Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits investigated, where the highest PCV and GCV values were recorded for ASV and GY, while the lowest values were recorded for AC, GL and GW (Table 5.2). The difference between genotypic and phenotypic coefficient of variation was small (<10.0) for all characters studied except for GY (Table 5.2). Broad sense heritability estimates (H^2) for grain yield and quality traits ranged from intermediate to high, varying from trait to trait (Table 5.2). The highest heritability was recorded for ASV, while GW indicated the lowest heritability estimate (Table 5.2). Genetic advance as a percent of mean was highest for GY followed by ASV, while the lowest was recorded for GW (Table 5.2).

Overall, the highest mean grain yield was recorded for 'Supa 1052 x NERICA 4' populations, followed by 'Komboka x NERICA 4' and 'Basmati 1052 x NERICA 4' populations (Table 5.2). Mean grain length was larger in 'Basmati 1052 x NERICA 4' populations, followed by

‘Komboka x NERICA 4’ and ‘Supa 1052 x NERICA 4’ populations (Table 5.2). Mean grain width was larger in ‘Basmati 1052 x NERICA 4’ populations, followed by ‘Supa 1052 x NERICA 4’ and ‘Komboka x NERICA 4’ populations (Table 5.2). Mean AC among the populations ranged from intermediate to high. Highest mean AC was recorded for ‘Basmati 1052 x NERICA 4’ populations and lowest for ‘Komboka x NERICA 4’ populations (Table 5.2). Mean ASV among the 3 populations were observed to be intermediate (Table 5.2).

Table 5.1: Mean squares and significant tests from analysis of variance of grain yield and quality traits among generations of parents, F$_2$ plants and BC$_1$F$_2$ individuals

Cross	Source of variation	d.f	GY	GL	GW	AC	ASV
‘Basmati 370 x NERICA 4’	Replication	2	1.296	0.276	0.090	41.420	0.219
	Seasons (S)	1	0.616	0.849	0.004	0.120	0.281
	Error a	2	0.542	0.051	0.027	65.000	0.219
	Populations (P)	2	1.933	0.117	0.032	9.050	0.281
	P x S	2	0.550	0.672	0.012	27.230	0.281
	Error b	8	0.882	0.308	0.020	33.730	0.422
	Generation (G)	3	5.860**	1.810***	0.277***	93.880**	9.013***
	G x S	3	0.265	0.266	0.010	76.920*	0.615
	G x P	6	1.319	0.070	0.028	26.220	0.221
	G x P x S	6	1.258	0.195	0.009	22.560	1.129
	Error c	36	1.164	0.236	0.030	21.000	0.558
‘Komboka x NERICA 4’	Replication	2	2.619	0.323	0.022	33.290	0.316
	Seasons (S)	1	2.262	0.156	0.008	2.780	0.587
	Error a	2	0.860	0.037	0.009	18.770	0.379
	Populations (P)	2	2.131	0.153	0.016	1.880	0.129
	P x S	2	0.152	0.116	0.002	29.080	0.045
	Error b	8	0.626	0.252	0.009	17.190	0.451
	Generation (G)	3	8.536***	1.160***	0.406***	262.650***	10.661***
	G x S	3	0.494	0.018	0.064	37.230*	0.291
	G x P	6	0.712	0.209	0.026	25.330	0.216
	G x P x S	6	1.180	0.336	0.027	24.980	0.568
	Error c	36	0.838	0.141	0.027	12.150	0.389
‘Supa 1052 x NERICA 4’	Replication	2	1.339	0.134	0.011	24.030	0.504
	Seasons (S)	1	0.259	0.000	0.004	3.990	0.517
	Error a	2	1.460	0.010	0.057	49.130	0.247
	Populations (P)	2	1.014	0.011	0.005	18.920	0.512
	P x S	2	0.173	0.121	0.049	43.640	0.109
	Error b	8	0.945	0.169	0.010	23.100	0.342
	Generation (G)	3	8.802***	3.141***	0.257***	204.010***	13.121***
	G x S	3	1.614	0.370	0.073	53.550**	0.243
	G x P	6	1.584	0.232	0.051	23.500	0.754
	G x P x S	6	0.517	0.083	0.025	22.190	0.418
	Error c	36	0.780	0.348	0.029	10.390	0.359

GY, grain yield (ton/ha); GL, grain length; GW, grain width; AC; amylose content; ASV, alkali spreading value ; *, **, ***, significant at P<0.05, P<0.01, P<0.001 respectively. S, seasons; P, populations; G, generations;

Table 5.2: Estimates of means, variability, heritability and genetic advance as percentage of mean for grain yield and quality traits among parents, F_2 plants and BC_1F_2 individuals

Cross	Character	Grand mean	PCV %	GCV%	D’	Heritability (H^2) (%)	Genetic advance (GA) as percent of mean
‘Basmati 370 x NERICA 4’	GY (t ha^{-1})	3.01	47.99	31.90	16.09	44.20	32.64
	GL (mm)	6.34	11.31	8.32	2.99	54.11	8.54
	GW (mm)	2.33	11.52	8.75	2.77	57.65	8.90
	AC %	26.72	18.03	5.57	12.46	9.55	3.37
	ASV	3.31	43.85	37.60	6.25	73.54	34.17
‘Komboka x NERICA 4’	GY (t ha^{-1})	3.01	49.91	39.57	10.34	62.86	39.41
	GL (mm)	5.78	10.28	7.96	2.32	59.94	8.03
	GW (mm)	2.27	12.75	10.53	2.22	68.23	10.10
	AC	25.68	27.44	23.85	3.59	75.53	21.12
	ASV	3.13	47.10	42.68	4.42	82.13	33.69
‘Supa 1052 x NERICA 4’	GY (t ha^{-1})	3.06	43.84	33.01	10.83	56.68	33.70
	GL (mm)	5.66	15.66	11.68	3.98	55.65	11.95
	GW (mm)	2.30	10.19	7.06	3.13	48.01	7.27
	AC	26.02	22.82	19.16	3.66	70.52	17.99
	ASV	3.47	45.09	41.65	3.44	85.33	30.36

GY, grain yield in tonnes per hectare; GL, grain length; GW, grain width; AC; amylose content; ASV, alkali spreading value ;PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; D’, difference between phenotypic and genotypic coefficients of variation; H^2, broad sense heritability;

5.3.3 Main-effect QTLs for yield-related and grain quality traits in F_2 populations

A total of 23 main-effect QTLs (M-QTLs) which individually explained between 0.6% and 54.8% of the total phenotypic variance (PV), were associated with yield-related and grain quality traits in 3 mapping F_2 populations (Table 5.3). Among these M-QTLs, thirteen were major QTLs (% PVE≥10) and ten were minor QTLs (% PVE<10). Population 1 (‘Basmati 370 x NERICA 4’) contributed 1 M-QTL while populations 2 (‘Komboka x NERICA 4’) and 3 (‘Supa 1052 x NERICA 4’) each had 11 M-QTLs. In addition, thirteen of the total number of M-QTLs identified had a negative additive effect, while the remaining 10 M-QTLs showed a positive additive effect. A summary of the M-QTLs detected for the traits investigated in this study is as follows;

Amylose content: Six M-QTLs for AC were identified on chromosomes 4,5,6,8 and 10 in F_2 mapping populations. One M-QTL was located in ‘Basmati 370 x NERICA 4’ F_2 population, two M-QTLs in ‘Komboka x NERICA 4’ F_2 population and three M-QTLs in ‘Supa 1052 x NERICA 4’ F_2 population. The M-QTL located in the ‘Supa 1052 x NERICA 4’ F_2 population within the marker interval SNP1872- SNP2172 on chromosome 10 had the greatest effect on AC with a PVE of 25.8% (Table 5.3 and Appendix 1).

Grain length: Five M-QTLs for GL were mapped on chromosomes 2,4,8,9 and 12. The M-QTL on chromosome 12 showed the largest effect, explaining 30.3% of the phenotypic variance in the ‘Komboka x NERICA 4’ F_2 population (Table 5.3 and Appendix 2).

Grain width: Seven M-QTLs for GW were identified on chromosomes 2, 3, 5, 7 and 8. Two M-QTLs on chromosomes 5 and 8 had the greatest effect on GW, explaining 45.8% and 54.8% of the phenotypic variance in the 'Komboka x NERICA 4' and 'Supa 1052 x NERICA 4' F_2 populations, respectively (Table 5.3 and Appendix 3).

Alkali spreading value: Four M-QTLs for ASV were located on chromosomes 1, 3, 5 and 12 in the 'Supa 1052 x NERICA 4' F_2 population. None of the identified ASV M-QTLs had marked effects on phenotypic variance, with PVE values of only 0.6–18.6 % (Table 5.3 and Appendix 4).

Table 5.3: Summary of main-effect QTLs detected by inclusive composite interval mapping (ICIM) for AC, GL, GW and ASV in 3 F_2 mapping populations

S/No	Population	QTL	Trait	Chr ł	Position	Left Marker	Right Marker	Minlog_{10}(P) ł‡	% PVE łł	Additive effect
1.	Basmati 370xNerica 4	*qAC-8-1*	AC	8	80.08	SNP13497	SNP13493	3.94	2.68	-1.50
2.	KombokaxNerica4	*qAC-4-2*	AC	4	50.35	SNP9066	SNP8771	1.87	1.91	-1.21
3.	KombokaxNerica4	*qAC-5-3*	AC	5	107.09	SNP10243	SNP10180	2.58	15.70	-3.47
4.	KombokaxNerica4	*qGL-2-1*	GL	2	142.44	SNP6083	SNP5718	1.78	1.17	-0.09
5.	KombokaxNerica4	*qGL-4-2*	GL	4	16.49	SNP9001	SNP8446	2.53	20.53	0.37
6.	KombokaxNerica4	*qGL-8-3*	GL	8	109.06	SNP13718	SNP13648	4.50	13.80	-0.30
7.	KombokaxNerica4	*qGL-9-4*	GL	9	9.59	SNP14993	SNP14813	1.98	5.22	-0.19
8.	KombokaxNerica4	*qGL-12-5*	GL	12	80.92	SNP4720	SNP4538	7.82	30.28	0.45
9.	KombokaxNerica4	*qGW-3-1*	GW	3	159.59	SNP7270	SNP7872	2.78	2.35	-0.04
10.	KombokaxNerica4	*qGW-5-2*	GW	5	81.66	SNP10248	SNP10095	3.06	1.38	0.03
11.	KombokaxNerica4	*qGW-5-3*	GW	5	40.16	SNP9942	SNP9944	6.55	45.76	0.16
12.	KombokaxNerica4	*qGW-7-4*	GW	7	121.04	SNP12543	SNP12618	5.43	16.97	0.10
13.	Supa1052xNerica4	*qAC-6-4*	AC	6	130.78	SNP11121	SNP11086	2.83	14.54	-3.12
14.	Supa1052xNerica4	*qAC-8-5*	AC	8	70.18	SNP13749	SNP13502	1.78	10.33	-2.63
15.	Supa1052xNerica4	*qAC-10-6*	AC	10	47.48	SNP1872	SNP2172	3.63	25.79	4.15
16.	Supa1052xNerica4	*qASV-1-1*	ASV	1	132.09	SNP374	SNP170	3.98	18.62	0.90
17.	Supa1052xNerica4	*qASV-5-2*	ASV	5	149.59	SNP7478	SNP7575	2.23	1.89	-0.29
18.	Supa1052xNerica4	*qASV-12-3*	ASV	12	20.97	SNP9995	SNP9948	2.62	8.67	0.61
19.	Supa1052xNerica4	*qASV-12-4*	ASV	12	85.6	SNP10068	SNP10288	2.33	0.56	-0.16
20.	Supa1052xNerica4	*qASV-3-5*	ASV	3	8.13	SNP4541	SNP4666	1.64	13.95	0.78
21.	Supa1052xNerica4	*qGW-2-5*	GW	2	81.92	SNP5943	SNP5818	2.35	4.31	-0.07
22.	Supa1052xNerica4	*qGW-5-6*	GW	5	2.62	SNP10045	SNP10147	3.39	25.76	0.17
23.	Supa1052xNerica4	*qGW-8-7*	GW	8	55.24	SNP13689	SNP13561	3.02	54.80	-0.25

ł Chr, Chromosome; łł %PVE, Percent phenotypic variation explained by a QTL; ł‡ Minlog_{10} (P), *P*-value

5.4 Discussion

Phenotypic variance was assessed for grain yield and quality traits for 3 different generations each consisting of two distinct parents, their F_2 population and BC_1F_2 individuals. Continuous phenotypic distributions and transgressive segregations in the variations for grain quality traits among the F_2 mapping populations indicated quantitative inheritance of grain length, grain width, amylose content and alkali spreading value in rice. Similar findings have been reported in other related studies (Lang and Buu, 2004; Lu et al., 2013; Dai et al., 2016). Highly significant differences ($p<0.01$ and $p<0.001$) for mean sums of squares among parents, F_2 plants, BC_1F_2 individuals for grain yield and quality traits suggested that sufficient amount of variability exists among the generations for the traits studied. Subsequently, this also revealed that selection will be effective when developing rice varieties with the desired qualities. Significant differences ($p<0.05$ and $p<0.01$) for the interaction between seasons and generations on amylose content indicated the influence of environment on the expression of this character. These results are in support of other previous studies by Fan et al. (2005) who detected environmental interactions for three main-effect QTLs for AC and Li et al. (2017) who concluded that genotype-by-environment interaction plays a significant role in determining starch traits such as AC.

PCV and GCV for all the studied characteristics revealed that there is great potential for selection of desirable traits from the developed populations for rice improvement. Selection within the characters with high PCV and GCV will be more effective than within those with low PCV and GCV. Grain yield and alkali spreading value indicated extremely high PCV and GCV values showing ample scope to select lines with improved grain yield and alkali spreading value. However, selection within the rest of the characters, particularly for grain appearance characteristics (grain length and width) and amylose content may not be effective as they recorded low PCV and GCV values. Low PCV and GCV values were also reported by Perera et al. (2014) for rice grain length and width. Accordingly, Perera et al. (2014) also noted that the extent of the environmental influence on any characteristic is specified by the magnitude of the difference between the PCV and GCV. In the present study difference between PCV and GCV was high for grain yield and amylose content (for progenies of the cross involving Basmati 370 x NERICA 4) indicating a marked influence of the environment on this character. Furthermore, small differences (<10.0) between the PCV and GCV for grain length, grain width and alkali spreading value among the parents and progenies of the 3 populations suggested presence of high genetic variability with less influence of the environment on these traits. Therefore, selection on the basis of phenotype alone can be effective for improvement of the aforementioned characters. Populations 'Supa 1052 x NERICA 4' and 'Komboka x NERICA

4’ recorded highest mean grain yield and intermediate AC and ASV. These crosses can be useful for selection of desirable segregates at the F_2 and BC_1F_2 generations.

Broad sense heritability estimates may not necessarily provide any indication of the degree of genetic progress that would result from selecting the best individuals since it includes both additive and non-additive effects (Perera et al., 2014). However, the use of broad sense heritability estimates accompanied with genetic advance provides a more reliable gauge of genetic progress (Ramanujam and Thirumalachar, 1967). Panse and Sukhatme (1967) indicated that traits that show high heritability with high genetic advance are controlled by additive gene action and therefore such characters can be improved through simple progeny selection methods (Babu et al., 2011). Thus, selection for characters that combine high heritability with high genetic advance is expected to accumulate more additive genes leading to further genetic improvement. Moderate to very high heritability values coupled with very high genetic advance as a percent of mean observed for alkali spreading value and grain yield suggested the preponderance of additive gene action in the expression of these traits. High heritability and genetic advance was reported by Sarawgi et al. (2000) for rice grain weight and by Vange (2009) for rice seed yield. Amylose content exhibited high heritability (for ‘Komboka x NERICA 4’ and ‘Supa 1052 x NERICA 4’ populations) coupled with moderate genetic advance, suggestive of predominance of non-additive gene action in the inheritance of this character with potential for improvement through selection. In a related study on the variability of AC in winter rice, Pathak et al. (2016) reported similar findings.

Quantitative trait loci analysis results demonstrated that some main-effects QTLs were consistently inherited based on their detection in different populations. The present study identified a total of 23 main-effect QTLs associated with yield-related and grain quality traits in 3 mapping F_2 populations. Complementary action of positive and negative effect of additive M-QTLs could well explain the genetic basis underlying transgressive segregation (Hu et al., 2013), where it is expected that favourable alleles for yield-related and grain quality traits are sparsely distributed within the two parents involved in the cross. Transgressive segregants have also been reported previously for yield-related and grain quality traits in rice (Septiningsih et al., 2003; Aluko et al., 2004; Yuan et al., 2010). Main- effects QTLs for AC were identified on chromosome 6, 8 and 10. Two main-effect QTLs for AC (*qAC-8-1* and *qAC-8-5)* identified on chromosome 8 came from the female parents (Basmati 370 and Supa 1052) and contributed to a negative additive effect and hence a decrease in AC. The position and nature of additive effect of the AC M-QTL differed from previous identified QTLs for amylose increase on the short and long arm of chromosome 8 that were reported by Takemoto-Kuno et al. (2015) and Li et al. (2011), respectively. Yacouba et al. (2013) also identified a QTL for AC with a positive additive effect on chromosome 8 in 2010 that accounted for 5.7% of the total phenotypic variation. This suggests that M-QTLs (*qAC-8-1* and *qAC-8-5)* detected in the present study are novel for low AC. Major QTLs with positive additive effect for grain length were identified on chromosomes 4 and 12 explaining 20.5% and 30.3% of phenotypic variance, respectively. Kato et al. (2011) also reported a major effect QTL with positive additive effect for grain length on chromosome 4 explaining 10.9% of phenotypic variance. However, the major QTL for grain length on chromosome 12 with positive additive effect has not been reported and is a potential novel QTL. A major QTL hotspot region with positive additive effect for grain width was identified consistently within the two mapping populations (Komboka x NERICA 4 and Supa 1052 x NERICA 4) on chromosome 5 explaining between 25.8% and 45.8% of phenotypic variance. The major QTL hotspot region for grain width has previously been mapped on chromosome 5 (Wan et al., 2008; Weng et al., 2008). Major effect QTLs for alkali spreading value detected on chromosomes 1 and 3 were previously reported by Kim and Kim (2016).

5.5 Conclusion

The present study highlighted that adequate genetic variability exists within the 3 mapping populations considered as revealed by significant variations for grain yield and quality traits. Hence single seed descent, pure line or pedigree selection methods can be effective to develop rice varieties with the desired qualities. However, the presence of genotype-by-environment interaction effects on grain yield and quality traits detected in this study is likely to substantially retard the breeding progress. The study suggests that traits which indicate smaller differences between the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) coupled with high heritability and genetic advance should be considered for direct selection. Thus, this would imply high genetic variability with less influence of the environment on the traits and therefore selection on the basis of phenotype alone can be effective. Also, relatively low differences (<10.0) between PCV and GCV were observed for grain length, grain width and alkali spreading value among parents and progenies of segregating populations. Moderate to high heritability values coupled with high genetic advance as a percent of mean were observed for alkali spreading value and grain yield. Consequently, the study recommends direct selection for grain length, grain width and alkali spreading value using single seed descent method in advanced F_2 and BC_1F_2 populations. Populations ‘Supa 1052 x NERICA 4’ and ‘Komboka x NERICA 4’ recorded the highest mean grain yield and intermediate AC and ASV. The aforementioned crosses also recorded the highest number (22) of main-effect QTLs (M-QTLs) detected among their F_2 mapping populations out of a total of 23 M-QTLs that were uncovered for grain length, grain width, amylose content and alkali spreading value. These crosses can be useful for selection of desirable segregates at the F_2 and BC_1F_2 generations. Each identified M-QTL individually explained between 0.6% and 54.8% of the total phenotypic variance. Both novel and previously reported main-effect QTLs were detected. Novel main-effect QTLs for low AC (*qAC-8-1* and *qAC-8-5)* were detected on chromosome 8 and accounted for 2.7% and 10.3% of the phenotypic variance, respectively. Further, a major QTL (*qGL-12-5)* for grain length with a positive additive effect, explaining 30.3% of phenotypic variance on chromosome 12 was identified as a potential novel QTL. The results of this study suggest that hybridization with *O. sativa* L. and interspecific hybrids can be successfully exploited to improve grain yield and quality in rice. Other main-effect QTLs for yield-related and grain quality traits such as; *qAC-6-4, qGL-4-2*, *qGW-5-3* and *qASV-3-5* that individually explained between 14% and 45.8% of the total phenotypic variance, were mapped to regions consistent with map locations in other studies and may be useful in marker-assisted selection experiments. Novel QTLs detected in this study for yield-related and grain quality traits could be considered as potential candidates for future fine-mapping and positional cloning projects.

References

Akhtar, S., M. Bhat, S.A. Wani, K. Bhat, S. Chalkoo, M. Mir and S.A. Wani. 2010. Marker Assisted Selection in Rice. Journal of Phytology. 2(10).

Allard, R.W. 1960. Principles of Plant Breeding.John Wiley and Sons Inc., New York, USA.pp 236.

Aluko, G., C. Martinez, J. Tohme, C. Castano, C. Bergman and J. Oard. 2004. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa*×*O. glaberrima*. Theoretical and Applied Genetics 109: 630-639.

Amarawathi, Y., R. Singh, A.K. Singh, V.P. Singh, T. Mohapatra, T.R. Sharma and N.K. Singh. 2008. Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Molecular Breeding 21: 49-65.

Asante, M.D. 2017. Breeding Rice for Improved Grain Quality. Advances in International Rice Research. InTech.

Babu, G., G. Lavanya and A. Singh. 2011. Genetic Variability for Grain Yield and Character Association Studies in Upland Rice(*Oryza sativa* L.) Germplasm. Environment and Ecology 29: 164-168.

Balasubramanian, V., M. Sie, R. Hijmans and K. Otsuka. 2007. Increasing rice production in Sub-Saharan Africa: Challenges and opportunities. Advances in Agronomy 94: 55-133.

Burton, G.W. 1952. Quantitative inheritance in grasses. Proceedings of 6th International Grassland Congress 1: 227-285.

Cruz, N.D. and G. Khush. 2000. Rice grain quality evaluation procedures. Aromatic Rices 3: 15-28.

Dai, L., L. Wang, Y. Leng, Y. Yang, L. Huang, L. Chen, Y. Wang, D. Ren, et al. 2016. Quantitative Trait Loci Mapping for Appearance Quality in Short-Grain Rice. Crop Science 56: 1484-1492.

Demont, M. 2013. Reversing urban bias in African rice markets: A review of 19 National Rice Development Strategies. Global Food Security. 2(3): 172-181.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. Plos One 6: e19379.

Fan, C., X. Yu, Y. Xing, C. Xu, L. Luo and Q. Zhang. 2005. The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. Theoretical and Applied Genetics. 110(8): 1445-1452.

Gibson, T., V. Solah and B. McCleary. 1997. A procedure to measure amylose in cereal starches and flours with concanavalin A. Journal of Cereal Science 25: 111-119.

Hu, W., M. Wen, Z. Han, C. Tan and Y. Xing. 2013. Scanning QTLs for grain shape using a whole genome SNP array in rice. Journal of Plant Biochemistry and Physiology. 1(1): 104.

IBP. 2015. "The IBP Breeding Management System Version 3.0.9 (December 2015). The Integrated Breeding Platform. https://www.integratedbreeding.net/breeding-management-system".

IRRI. 2002. The standard evaluation system for rice reference manual. International Rice Research Institute, Los Banos, Philippines. doi:www.knowledgebank.irri.org/extension/index.php/ses.

Jing, Z., Y. Qu, C. Yu, D. Pan, Z. Fan, J. Chen and C. Li. 2010. QTL analysis of yield-related traits using an advanced backcross population derived from common wild rice (*Oryza rufipogon L*). Molecular Plant Breeding. Vol 1.No.1.

Johnson, H.W., H. Robinson and R. Comstock. 1955. Estimates of genetic and environmental variability in soybeans. Agronomy Journal 47: 314-318.

Kato, T., S. Segami, M. Toriyama, I. Kono, T. Ando, M. Yano, H. Kitano, K. Miura, et al. 2011. Detection of QTLs for grain length from large grain rice (*Oryza sativa* L.). Breeding Science 61: 269-274.

Kim, H.Y. and K.M. Kim. 2016. Mapping of grain alkali digestion trait using a Cheongcheong/Nagdong doubled haploid population in rice. Journal of Plant Biotechnology 43: 76-81.

Kosambi, D. 1944. The estimation of map distances from recombination values. Annals of Eugenics 12: 172-175.

Lang, N.T. and B.C. Buu. 2004. Quantitative analysis on amylose content by DNA markers through backcross populations of rice (*Oryza sativa* L.). OMonRice 12: 12-17.

Li, J., W. Zhang, H. Wu, T. Guo, X. Liu, X. Wan, J. Jin, T.T.T. Hanh, et al. 2011. Fine mapping of stable QTLs related to eating quality in rice (*Oryza sativa* L.) by CSSLs harboring small target chromosomal segments. Breeding Science 61: 338-346.

Li, K., J. Bao, H. Corke and M. Sun. 2017. Genotypic diversity and environmental stability of starch physicochemical properties in the USDA rice mini-core collection. Food Chemistry 221: 1186-1196.

Little, R.R., G.B. Hilder and E.H. Dawson. 1958. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry. 35(2): 111-126. .

Liu, Q.M., J.H. Jiang, F.A. Niu, Y.J. He and D.I. Hong. 2013. QTL analysis for seven quality traits of RIL population in *Japonica* rice based on three genetic statistical models. Rice Science 20: 31-38.

Lou, J., L. Chen, G. Yue, Q. Lou, H. Mei, L. Xiong and L. Luo. 2009. QTL mapping of grain quality traits in rice. Journal of Cereal Science 50: 145-151.

Lu, B.Y., C.Y. Yang, K. Xie, L. Zhang, T. Wu, L.F. Li, X. Liu, L. Jiang, et al. 2013. Quantitative trait loci for grain-quality traits across a rice F-2 population and backcross inbred lines. Euphytica 192: 25-35.

Lush, J.L. 1949. Heritability of quantitative characters in farm animals. Hereditas 35: 356-375.

Maas, L.F., A. McClung and S. McCouch. 2010. Dissection of a QTL reveals an adaptive, interacting gene complex associated with transgressive variation for flowering time in rice. Theoretical and Applied Genetics. 120(5): 895-908.

Mccouch, S.R., Y.G. Cho, M. Yano, E. Paul, M. Blinstrub, H. Morishima and T. Kinoshita. 1997. Report on QTL nomenclature. Rice Genetics Newsletter 14: 11-131.

Moncada, P., C. Martinez, J. Borrero, M. Chatel, H. Gauch Jr, E. Guimaraes, J. Tohme and S. McCouch. 2001. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theoretical and Applied Genetics 102: 41-52.

Panse, V. and P. Sukhatme. 1967. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research.New Delhi (India).

Pathak, K., S. Rathi, H. Verma, R.N. Sarma and S. Baishya. 2016. Variability in Grain Quality Characters of Local Winter (Sali) Rice of Assam, India. Indian Journal of Plant Genetic Resources 29: 22-31.

Perera, U., D. Ratnasekera, S. Senanayake and D.S. de Z Abeysiriwardena. 2014. Genetic parameters and correlations of yield attributing characteristics of weedy rice in Sri Lanka. Journal of the National Science Foundation of Sri Lanka 42.

Ramanujam, S. and D. Thirumalachar. 1967. Genetic variability of certain characters in red pepper (*Capsicum annum* L.). Mysore Journal of Agricultural Science 1: 30-36.

Sarawgi, A., N. Rastogi and D. Soni. 2000. Studies on some quality parameters of indigenous rice in Madhya Pradesh. Annals of Agricultural Research 21: 258-261.

Seck, P.A., E. Tollens, M.C.S. Wopereis, A. Diagne and I. Bamba. 2010. Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. Food Policy 35: 403-411.

Septiningsih, E., J. Prasetiyono, E. Lubis, T. Tai, T. Tjubaryat, S. Moeljopawiro and S. McCouch. 2003. Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theoretical and Applied Genetics 107: 1419-1432.

Sreenivasulu, N., V.M. Butardo Jr, G. Misra, R.P. Cuevas, R. Anacleto and P.B. Kavi Kishor. 2015. Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. Journal of Experimental Botany 66: 1737-1748.

Swamy, B., K. Kaladhar, N. Shobha Rani, G.S. Prasad, B. Viraktamath, G. Reddy and N. Sarla. 2012. QTL analysis for grain quality traits in 2 BC2F2 populations derived from crosses between *Oryza sativa* cv Swarna and 2 accessions of *O. nivara*. Journal of Heredity. 103(3): 442-452.

Takemoto-Kuno, Y., H. Mitsueda, K. Suzuki, H. Hirabayashi, O. Ideta, N. Aoki, T. Umemoto, T. Ishii, et al. 2015. qAC2, a novel QTL that interacts with Wx and controls the low amylose content in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 128: 563-573.

Tan, Y., J. Li, S. Yu, Y. Xing, C. Xu and Q. Zhang. 1999. The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theoretical and Applied Genetics. 99(3-4): 642-648.

Tyagi, S., R. Mir, H. Kaur, P. Chhuneja, B. Ramesh, H. Balyan and P. Gupta. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). Molecular Breeding. 34(1): 167-175.

Vange, T. 2009. Biometrical studies on genetic diversity of some upland rice (*Oryza sativa* L.) accessions. Nature and Science 7: 21-27.

VSN, I. 2011. GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

Wan, X., J. Weng, H. Zhai, J. Wang, C. Lei, X. Liu, T. Guo, L. Jiang, et al. 2008. Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele gw-5 in a recombination hotspot region on chromosome 5. Genetics 179: 2239-2252.

Wang, L., W. Liu, Y. Xu, Y. He, L. Luo, Y. Xing, C. Xu and Q. Zhang. 2007. Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain. Theoretical and Applied Genetics. 115(4): 463-476.

Wang, P., Y. Xing, Z. Li and S. Yu. 2012a. Improving rice yield and quality by QTL pyramiding. Molecular Breeding 29: 903-913.

Wang, S., K. Wu, Q. Yuan, X. Liu, Z. Liu, X. Lin, R. Zeng, H. Zhu, et al. 2012b. Control of grain size, shape and quality by OsSPL16 in rice. Nature Genetics 44: 950.

Weng, J., S. Gu, X. Wan, H. Gao, T. Guo, N. Su, C. Lei, X. Zhang, et al. 2008. Isolation and initial characterization of *GW5,* a major QTL associated with rice grain width and weight. Cell Research 18: 1199.

Xie, X., F. Jin, M. Song, J. Suh, H. Hwang, Y. Kim, S. McCouch and S. Ahn. 2008. Fine mapping of a yield enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* x *O. rufipogon* cross. Theoretical and Applied Genetics. 116: 613-622.

Xu, Y. and J.H. Crouch. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Science 48: 391-407.

Yacouba, N.T., B. Yu, G. Gao, Q. Zhang, C. Xu, J. Xiao and Y. He. 2013. QTL analysis of eating quality and cooking process of rice using a new RIL population derived from a cross between Minghui 63 and Khao Dawk Mali105. Australian Journal of Crop Science 7: 2036.

Yamamoto, T., J. Yonemaru and M. Yano. 2009. Towards the understanding of complex traits in rice: substantially or superficially? DNA Research. 16(3): 141-154.

Yi, M., K.T. Nwe, A. Vanavichit, W. Chai-arree and T. Toojinda. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113: 178-186.

Yuan, P.R., H.J. Kim, Q.H. Chen, H.G. Ju, S.D. Ji and S.N. Ahn. 2010. Mapping QTLs for grain quality using an introgression line population from a cross between *Oryza sativa* and *O. rufipogon*. Journal of Crop Science and Biotechnology 13: 205-212.

Zhao, X., L. Zhou, K. Ponce and G. Ye. 2015. The Usefulness of Known Genes/Qtls for Grain Quality Traits in an Indica Population of Diverse Breeding Lines Tested using Association Analysis. Rice 8: 1.

Zhou, L., L. Jiang, H. Zhai and J. Wan. 2009. Current status and strategies for improvement of rice grain chalkiness. Hereditas (Beijing) 31: 563-572.

6. General overview and implications of the study

6.1 Introduction and objectives of the study

Rice (*Oryza sativa* L.) is the staple food for over half of the world's population and the fourth most important and widely cultivated cereal crop in sub-Sahara Africa (SSA). Despite sustained initiatives to encourage local rice production by the rice sector in SSA and particularly in South Sudan, production is still low and unable to meet the growing demand for high quality rice. Improving grain yield and quality are important considerations but present a major challenge for most rice breeding programs in sub-Saharan Africa. In this regard, the research focus was to generate a comprehensive understanding of the genetic basis of yield-related and grain quality traits in rice as an important step towards formulating effective breeding strategies. Consequently, this research focus was used to formulate the objectives of the present study and the hypotheses that were to be tested. This chapter gives an overview of the whole study by reiterating on the major findings, their implications and the way forward for future research. The objectives of this study were:

v. To investigate farmers' perceived rice production and productivity constraints and choice of rice ideotypes across rainfed and irrigated rice agro-ecologies in South Sudan, to guide breeding and policy interventions.

vi. To perform genetic and comparative analysis of agro-morphological and grain quality traits of elite upland rice genotypes.

vii. To investigate genetic diversity and perform genome-wide association studies of grain quality traits in a panel of 59 upland and lowland rice collections.

viii. To determine genetic variability and identify QTLs for yield-related and grain quality traits in three rice F_2 populations involving crosses of *O.sativa* L. and an interspecific hybrid (*O. sativa* L. x *O. glaberrima,* Steud).

6.2 Summary of the major findings

Appraisal of major determinants of rice production and productivity, and farmers' choice of rice ideotypes in South Sudan: implications for breeding and policy interventions

A survey study was conducted targeting five payams (administrative units) including Aweil Rice Scheme, Yambio centre, Bangasu, Gangura and Lirangu payams that reflect major rice growing areas under rainfed and irrigated cultivation in South Sudan. The main findings were;

- Common rice landraces were 'BG400-1' and 'BR4' for lowland rice ecology and 'Ruanya' and 'Zamburu' under upland rice ecologies. Estimate yields of the landraces ranged between 0.4 t ha^{-1} and 1.6 t ha^{-1}.
- Farmers mainly cultivated local rice landraces due to inadequate exposure to new and modern cultivars or the absence of acceptable alternatives to their landraces.

- Imported rice varieties were highly preferred mainly for the “sweet” and appealing taste, grain shape and size, aroma, swelling capacity and non-stickiness during cooking, while the locally produced rice cultivars were less preferred due to their non-appealing grain shape and size, taste and stickiness during cooking.
- The best ranked upland rice cultivars were ‘NERICA 1’ and ‘ART3-8L6P3-2-3-B’, which were selected for early maturity and enhanced tillering capacity.
- The best two selected lowland cultivars were ‘NERICA-L-6’ and ‘K-8’. They were most preferred for early maturity, large panicles and enhanced tillering capacity.
- Significant differences ($P<0.001$) were observed in the consistency of ranking order of major rice production constraints across lowland and upland rice growing sites.
- Poor soil and water management practices was identified as a major constraint to lowland rice production, while unreliable rainfall was mentioned as a critical factor affecting upland rice production.

Genetic analysis of elite upland rice genotypes using DArTseq markers and comparative analysis of agro-morphological and grain quality traits

The study was undertaken to assess the genetic diversity and relationship present among 36 elite upland rice genotypes using DArTseq-derived SNP markers and agro-morphological and grain quality traits. The main outcomes were;

- Grain yield (GY) was highest in genotype ‘ART10-1L15P1-4-3-1’ (5.1 t ha^{-1}) and lowest in ‘WAC x NERICA 4’ (1.6 t ha^{-1}).
- Number of grains per panicle expressed the greatest maximum direct positive effect (0.715) on grain yield.
- Plant height exhibited the largest negative direct (-0.146) effect on grain yield.
- Grain length (GL) exhibited the greatest maximum direct positive effect on amylose content (AC) (0.471), while grain width (GW) revealed the largest negative effect on AC (-0.862).
- Upland rice genotypes that include; P5H6, NAMCHE 6 and ART3-7L9P8-3-5-B-B-2, had considerable high yields and intermediate values for AC and alkaline spreading value (ASV).
- The most significant peak of ΔK from STRUCTURE run was observed when $K=2$, thus, the entire set of upland genotypes were grouped into two major populations based on environmental adaptations and breeding history.

Diversity analysis and genome-wide association studies of grain quality traits in rice (*Oryza sativa* L.) using DArTseq markers

The present study performed genetic diversity and genome-wide association studies (GWAS) of grain quality traits in a diverse collection of 59 upland and lowland rice (*Oryza sativa* L.) genotypes. Genotypes were acquired from AfricaRice (ARC), National Crops Resources Research Institute, Uganda (UG), Institut d'Economie Rurale, Mali (IER), International Rice Research Institute (IRRI), South Sudan landraces (LDR), Madagascar (MDG), Tanzania (TZ) and International Centre for Tropical Agriculture (CIAT). The major findings were;

- The tendencies of polymorphism information content (PIC) and mean number of alleles were in the order ARC=UG > IRRI > IER > LDR > MDG > TZ > CIAT.
- The most significant peak of ΔK from STRUCTURE run was observed when K=2, hence, the entire set of upland and lowland rice genotypes were grouped into two major populations based on environmental adaptations and breeding history.
- Only genotypes with origin from UG suggested considerable degree of admixtures (<80%).
- The greatest genetic distance was observed between genotypes from TZ and CIAT populations (0.865) and between genotypes from TZ and MDG populations (0.808).
- Analysis of the frequency distributions of phenotypic classes of grain quality traits among test genotypes suggested that all traits were quantitative and continuous.
- A total of 22 significant (P < 0.001) association signals were detected for grain quality traits, among which 20 significant SNP loci represented potential novel QTLs.
- SNP12_100006178, SNP13_3052560 and SNP14_3057360 individually co-localised with two functional gene groups that were associated with QTLs for grain width (GW) and grain length to width ratio (L/W) on chromosome 3, indicating trait dependency or pleiotropic-effect loci.
- Significant associations for AC allele (C/T) was traced back to parent K5; ASV alleles (G/A, A/G) were located in parents 'ART2-4L3P1-2-1', 'BG400-1', 'JARIBU' and 'SUPA TZ'; while the co-localised QTLs for GW and L/W came from 'JARIBU', 'BR4' and 'ART3-8L6P3-2-2-B'.

Genetic variability and quantitative trait loci (QTL) analysis for yield-related and grain quality traits in three rice F_2 populations

The main objective of this study was to map QTLs for yield-related and grain quality traits of rice using three F_2 populations derived from the cross between *O. sativa* L. and an interspecific hybrid rice cultivar (O*. sativa* L. x *O. glaberrima,* Steud). The key findings were;

- Mapping populations showed a continuous and normal distribution for grain quality traits with transgressive segregations in variations.
- Analyses of variance among generations involving parents, F_2 populations and BC_1F_2 individuals consistently demonstrated highly significant differences (p<0.01 and p<0.001) for grain yield (GY) and quality traits.
- Significant differences (p<0.05 and p<0.01) were observed for the interaction between seasons and generations.
- Highest phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values were recorded for GY and ASV, while the lowest values were recorded for AC, GL

and GW. The difference between PCV and GCV was small (<10.0) for all characters studied except for GY.

- Broad sense heritability estimates (H^2) for grain yield and quality traits ranged from low to intermediate and high, but varied considerably from trait to trait with the highest recorded for ASV and lowest for AC and GL.
- Genetic advance was highest for GY followed by ASV. Amylose content recorded the lowest genetic advance.
- A total of 23 main-effect QTLs (M-QTLs) associated with yield-related and grain quality traits were identified for AC on chromosome 6, 8 and 10, with those on chromosome 8 (*qAC-8-1* and *qAC-8-5)* differing from previously identified QTLs suggesting that the QTLs are novel for low AC.
- Major QTLs with positive additive effect for grain length were identified on chromosomes 4 and 12 explaining 20.5% and 30.3% of phenotypic variance (PV), respectively. The major QTL for GL on chromosome 12 with positive additive effect has not been reported and is a potential novel QTL.
- A major QTL hotspot region with positive additive effect for GW was identified on chromosome 5 explaining between 25.8% and 45.8% of PV consistent with previous studies. Major effect QTLs for ASV detected on chromosomes 1 and 3 had previously been reported in related studies.
- Populations ‘Supa 1052 x NERICA 4’ and ‘Komboka x NERICA 4’ recorded the highest mean grain yield and intermediate AC and ASV, as well as the highest number (22) of detected main-effect QTLs (M-QTLs) in their F_2 populations.

6.3 Implications of the study and way forward

Participatory plant breeding approaches including participatory rural appraisals (PRA) have been widely embraced in plant breeding programs to engage farmers in variety selection process and have proven to be successful in eliciting farmers’ preferred varieties and encouraging farmer variety adoption. In addition, participatory rural appraisal has been useful in identifying farmers’ crop production constraints and traits that farmers value in the selection of rice cultivars to provide important considerations for goal setting in a plant breeding program. The results of this study show a need for integration of farmers’ and other important stakeholders’ preferences in variety development process and the desirability of releasing site-specific rice cultivars given the differences in bio-physical, socio-cultural and farmers’ preferences across major rice growing areas of South Sudan. The study further recognizes the important role of research in generating appropriate rice technologies while advocating for policy measures that encourage quality rice seed production and distribution in the country.

Genetic variability has been a major driving force in selecting for superior genotypes in crop improvement programs, while knowledge on variability and association between yield attributing factors and other important agro-morphological and grain quality traits is critical in tailoring hybridization programs. Further, the magnitude of genetic variability and the extent to which the desirable characters are heritable largely determines the success of any plant breeding program. Additionally, association mapping based on phenotypic and genotypic data has been critical in identifying molecular markers or QTLs linked to traits of interest and with potential for use in marker-assisted breeding (MAS). Furthermore, mapping of QTLs for yield-related and grain quality traits in rice is an important genetic approach to dissect and integrate them in MAS and for gene discovery.

The present study observed considerable genetic variations for agro-morphological characters and grain quality traits among 36 upland rice genotypes. The study further revealed that wide variability for grain quality traits exists within the current lowland and upland rice germplasm collection which can be exploited for production and rice improvement for grain yield and quality. A low degree of differentiation among sampled populations suggested the need for widening the genetic base through the introduction of distant or wild relatives.

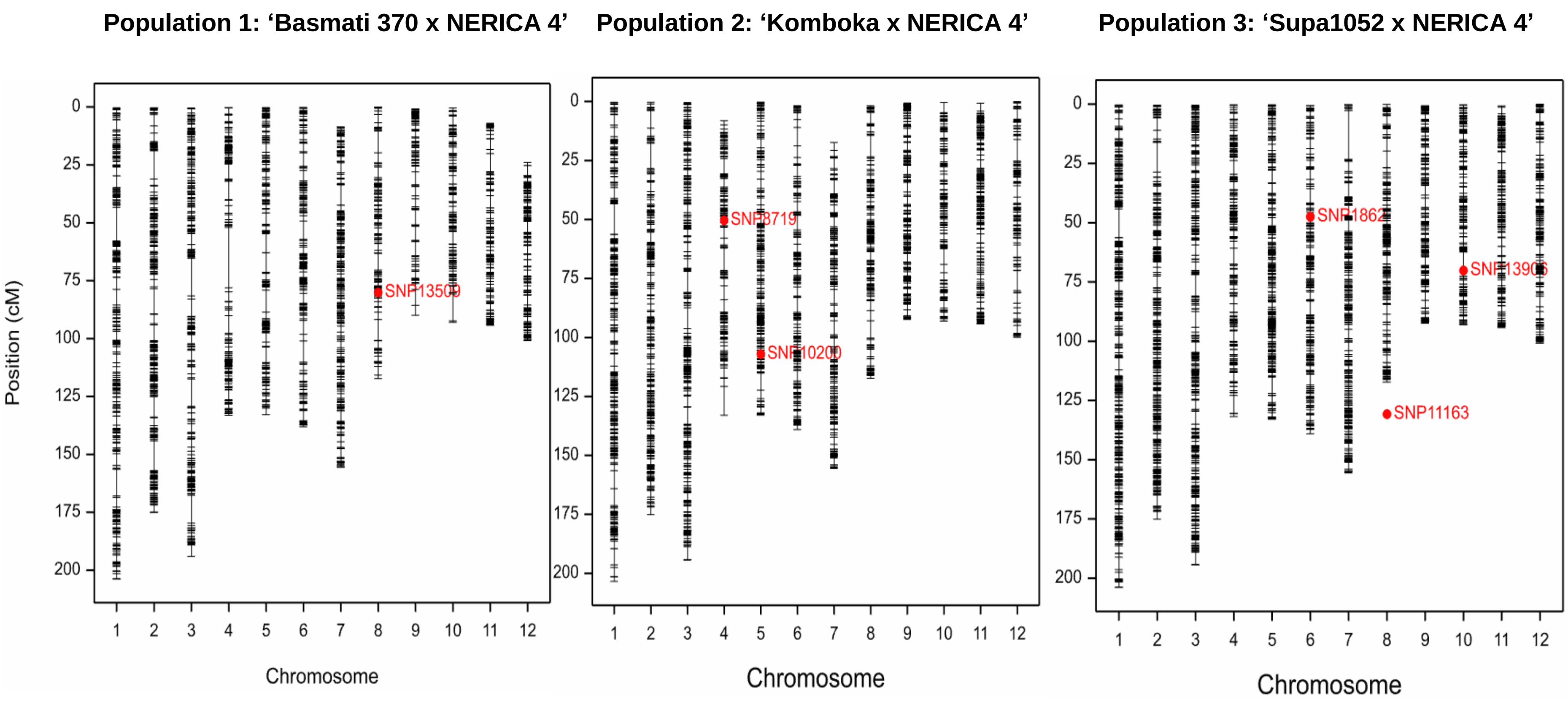
Plausible associations identified for yield and grain quality attributing factors are important selection criteria for enhancing yield and grain quality in rice. The DArTseq-derived SNP markers and agro-morphological and grain quality traits managed to identify upland rice genotypes: ‘P5H6’, ‘NAMCHE 6’ and ‘ART3-7L9P8-3-5-B-B-2’ that can be used for direct production or breeding.

GWAS indicated presence of novel alleles associated with rice grain quality traits and significant QTL associations for AC allele (C/T) that can be traced back to parent K5; ASV alleles (G/A, A/G) were located in parents ‘ART2-4L3P1-2-1’, ‘BG400-1’, ‘JARIBU’ and ‘SUPA TZ’; while the co-localised QTLs for GW and L/W came from ‘JARIBU’, ‘BR4’ and ‘ART3-8L6P3-2-2-B’. These parents are potential sources of major effect QTLs for grain quality and hence can be exploited for rice improvement. The study revealed the potential of highly informative and selective DArTseq-derived SNP markers for genetic diversity analysis and GWAS in rice.

The study further highlighted adequate genetic variability within the 3 mapping populations for grain yield and quality traits indicating that single seed descent, pure line or pedigree selection methods can be effective to develop rice varieties with the desired qualities. However, the presence of genotype-by-environment interaction effects on grain yield and quality traits detected in the study is likely to substantially retard the breeding progress. The study suggests that traits which indicate smaller differences between the phenotypic coefficient of variation and genotypic coefficient of variation coupled with high heritability and genetic advance should be considered for direct selection. Thus, this would imply high genetic variability with less influence of the environment on the traits and therefore selection on the basis of phenotype alone can be effective. Consequently, the study recommends direct selection for grain length, grain width and alkali spreading value using single seed descent method in advanced F_2 and BC_1F_2 populations. Populations ‘Supa 1052 x NERICA 4’ and ‘Komboka x NERICA 4’ which recorded the highest mean grain yield and intermediate AC and ASV, can be useful for selection of desirable segregates at the F_2 and BC_1F_2 generations.

Detection of novel QTLs that enhanced grain quality underscores the potential value of the germplasm used in the present study as a useful source for grain yield and quality improvement. Main-effect QTLs that mapped to regions consistent with map locations in other studies may be useful in MAS experiments. Novel QTLs identified in this study for yield-related and grain quality traits could serve as potential candidates for future fine-mapping and positional cloning projects.

Appendices:

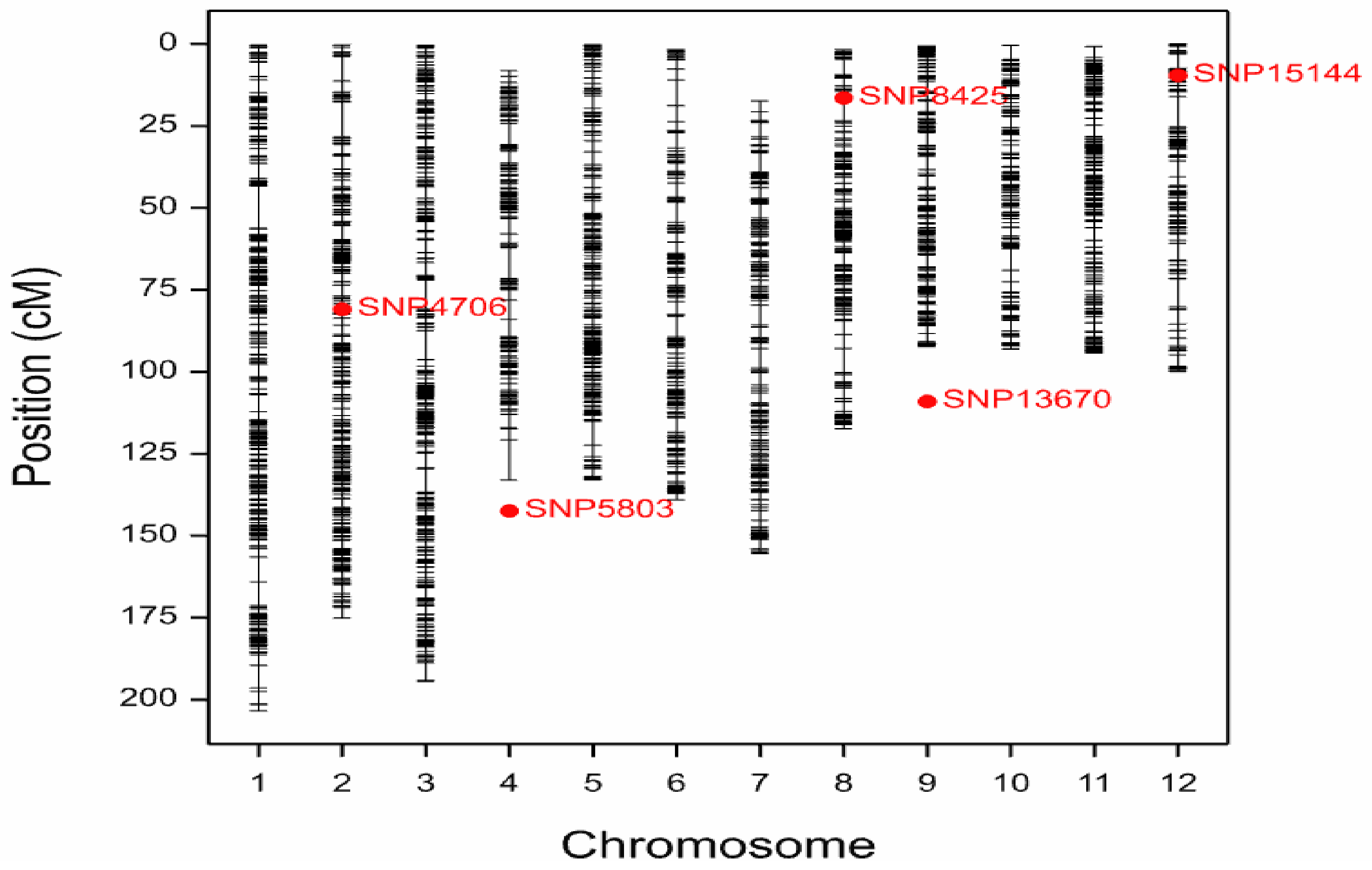


Population 1: ‘Basmati 370 x NERICA 4’

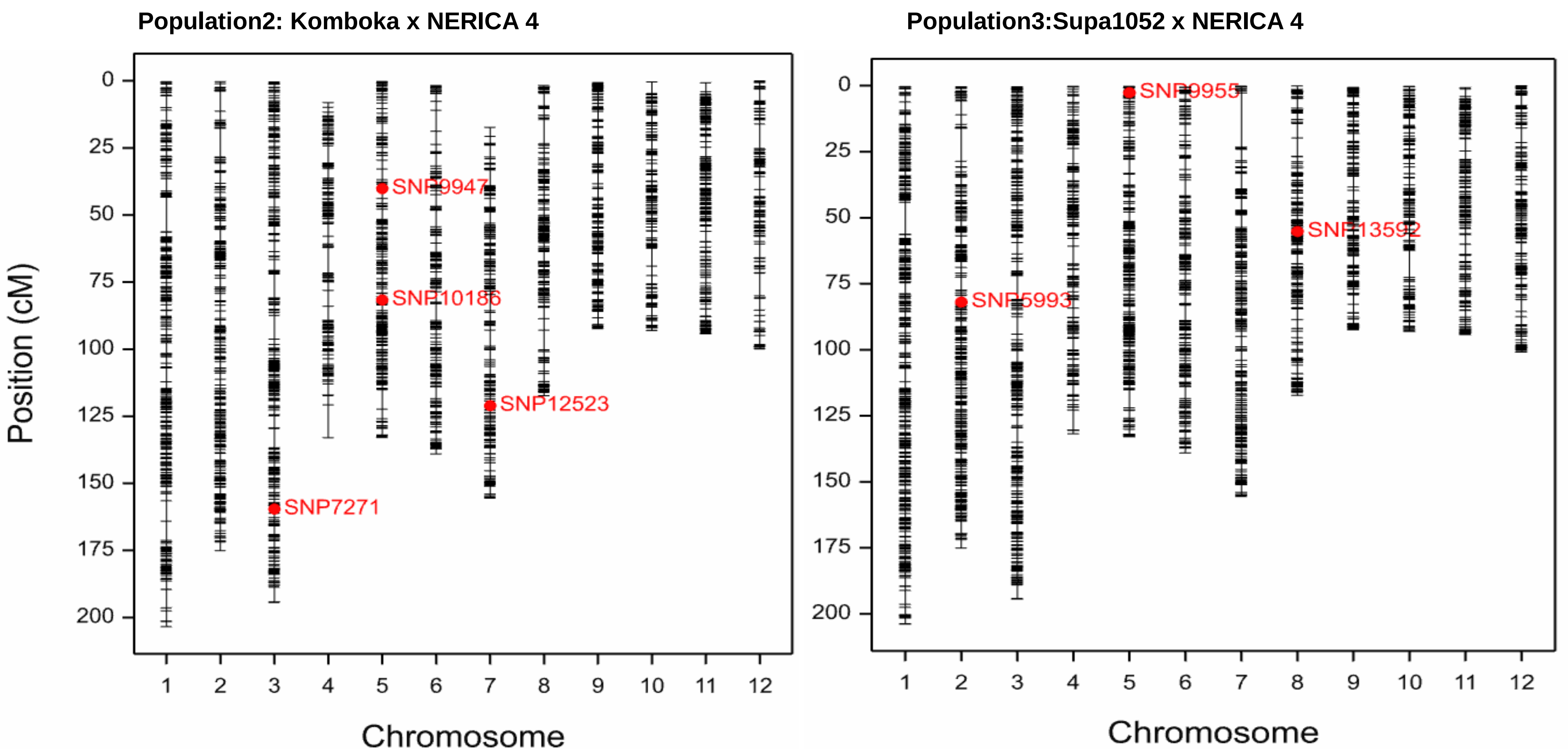
Population 2: ‘Komboka x NERICA 4’

Population 3: ‘Supa1052 x NERICA 4’

Appendix 1: Position of DArTseq-derived SNP markers and amylose content (AC) QTLs in linkage map of 3 mapping populations. QTLs positions are shown by red colour on identified linkage groups, while map distances are indicated on the left side of the linkage groups.

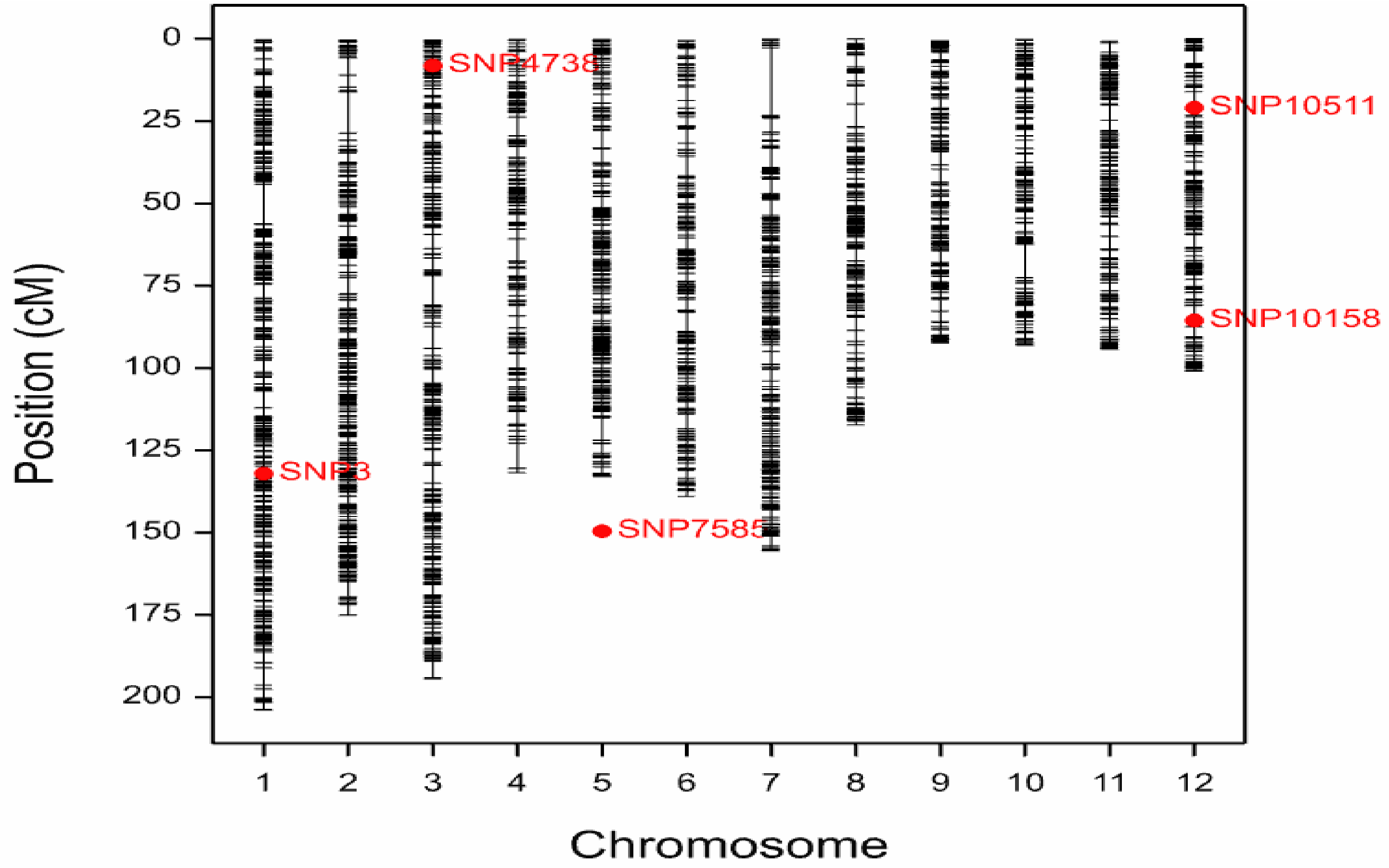


Appendix 2: Position of DArTseq-derived SNP markers and grain length (GL) QTLs in linkage map of population 2 (‘Komboka x NERICA 4’). QTLs positions are shown by red colour on identified linkage groups, while map distances are indicated on the left side of the linkage groups.


Population2: Komboka x NERICA 4

Population3:Supa1052 x NERICA 4

Appendix 3: Position of DArTseq-derived SNP markers and Grain width (GW) QTLs in linkage map of populations 2 and 3. QTLs positions for GL are shown by red colour on identified linkage groups, while map distances are indicated on the left side of the linkage groups.



Appendix 4: Position of DArTseq-derived SNP markers and alkali spreading value (ASV) QTLs in linkage map of population 3 (‘Supa1052 x NERICA 4’). QTLs positions are shown by red colour on identified linkage groups, while Map distances are indicated on the left side of the linkage groups

Appendix 5: **Rice baseline survey data collection protocols**

Individual farmer's questionnaire

Section A: General information

Name of enumerator ………………………….. Date of interview ………………
Country …………………………State ……………………County …………………………
Payam ……….........Boma …………………………………………….Village
……………………………………………………..…………………
Name of farmer ………………………………………………………………………………
Relationship to household head ……………. **(1=Head, 2=Spouse, 3=Child, 4=Relative)**

Section B: Household and socio-economic characteristics

1. Age of farmer in years…………………………………………………………………………………………
………………
2. Gender of farmer (**1=male, 2=female)**
…..
3. Highest formal education **(circle the right codes)**
1=None; 2=primary; 3=Ordinary secondary; 4=Advanced secondary; 5=Certificate graduate; 6=Diploma graduate; 7=Degree graduate; 8=other (specify)
…………………………………………………….
4. Household size
………………………………………………………………………………………………………
…………………
5. Total land/farm size (acres/ feddans)
……………………………………………………………………………………….
6. Land under rice (acres/ feddans)
…………………………………………………………….....……………………….
7. State the income generating activities that you are involved in **(circle the right codes)**
Crop farming=1; livestock production=2; trade=3; handicraft=4; labourer=5; formal employment=6; other (specify)………………………………………………………………………………..
……………………………………

Section C: Rice Production

1. How long have you been involved in rice production (years)?
…………………………………………………………
2. Please state the varieties of rice you grow and area under each (use table below)? **(Incase farmer did not grow rice in 2011 but did so this year, take note of data for this year as a foot note below the page)**

Rice variety*	Ecology**	Seed source***	Area under the variety (acres/feddans)	
			1st season 2014	2nd season 2014

***Variety codes:1=Supa; 2=Kaiso; 3=Superica 1; 4=Nerica 4/Naric 3/Superica 2; 5=Nerica 1; 6=Nerica 10; 7=Nerica lowland; 8=Naric 1 ; 9=Naric 2 ; 10=Sindano; 11=IRAT, 12=TOX 5, 13=other traditional varieties (specify, e.g. Pakistani); 14=other (specify); 99=don't know variety**
**** Ecology codes : 1=upland ; 2=lowland ; 3=irrigated**
*****Seed source codes : 1=own seed ; 2=fellow farmer ; 3=local market ; 4=NGOs ; other (specify)**

3. Which variety is most preferred by the farmer? And why?
……………………………….…………………………

…………………………………………………………..…………………………………………………
……
…………………………………………………………………………………………..…………

4. Which variety is most preferred in the market? And Why?

……
……
………………………………………………………………………………………………………

5. Which variety is least preferred by the farmer? And why?

……
……
……………………………………………………………………………………………………..

6. Which variety is least preferred in the market? And why?

…………………………………………..…………………………..
……
………
……

7. Please state rice production inputs, their sources and costs, and constraints associated with these inputs

Type of input	**Input name**	**Input source***	**Quantity used**	**Unit (1=kg, 2=litres)**	**Land size and unit (1=acre; 2=task)****	**Cost per unit**	**Input acquisition Problems**
Fertilizer							
Insecticide							
Fungicide							
Credit							
Seed (by variety)							
Hired labour							

*** Input source codes: 1=own saved; 2=fellow farmer; 3=local market; 4=stockist; 5=input dealer; 6=NGO; 7=other (specify)**

****Incase of task; define the task in form of (size i.e. width -----------by length---------------) and the measurement unit of the task stick e.g 5m**

8. Who offers you technical skills and information in rice production? **Codes: 1=own experience; 2=fellow farmer; 3=extension ; 4=NARS; 5=NGOs ; 6=farmer organization ,7=other (specify)….**

……………………………………………………………………………….……………………
……………………………………………..

9. Please state any other support and its source that you obtained in line with rice production

Type of Support*	Source of support	Quantity

*** Type of support can be in terms of seeds, fertilizer, credit, chemicals, etc.**

10. Please state any arrangements (informal, partnerships, contracts) that you are a part of; for rice production

Name of the arrangement	Activities under arrangement	Benefits you receive from the arrangement	Challenges

11. Specify rice production and sales in 2014

Season 1	Area (acres/ feddans)	Production (kgs)		Quantity sold (kgs)			Selling price/kg		
Variety*		Grain	Seed	Paddy	Milled**	Seed	Paddy	Milled	Seed
Season 2									

***Variety codes:1=Supa; 2=Kaiso; 3=Superica 1; 4=Nerica 4/Naric 3/Superica 2; 5=Nerica 1; 6=Nerica 10; 7=Nerica lowland; 8=Naric 1 ; 9=Naric 2 ; 10=Sindano; 11=IRAT, 12=TOX 5, 13=other traditional varieties (specify, e.g. Pakistani); 14=other (specify); 99=don't know variety**

****Define milling recovery (for every 1kg of paddy milled, what is the quantity of milled rice resulting from it)**

12. State any constraints associated with rice production that you faced

Constraints (5 main ones)	Rank	Coping mechanism	Source of coping mechanism information*	Suggested solution**

***Coping mechanism refers to the way the farmer is dealing with the problem in question**
****Suggested solution refers to ways that the farmer thinks the problem in question could be dealt with but he/she is not using it.**
Codes for source of coping mechanism: 1=own experience; 2=fellow farmer; 3=extension; 4=NGOs; 5=NARS; 6=farmer organization; 7=other (specify)

13. List farmer's preferred rice variety traits and their level of acceptance

Trait	Score ɫ	Overall mean	
		Score	Rank ǂ
Yield			
Early maturity			
Cooking and eating quality			
Nutritional importance			
Drought tolerance			
Pest and insect resistant			
Phenotypic acceptability			
Disease resistance			

Codes: 1=Nutritional importance; 2= Early maturity; 3= Drought tolerance; 4= Pest/insect resistant; 5= Disease resistant; 6= Yield; 7= Cooking and eating quality; 8= Phenotypic acceptability

14. List farmer's preferences for staple crops in order of importance

Crop	Score ɫ	Overall mean	
		Score	Rank ǂ
Sorghum			
Cassava			
Maize			
Groundnut			
Beans			
Sesame			
Millet			
Rice			

Codes: 1=Sorghum; 2= Cassava; 3= Maize; 4= Groundnut; 5= Beans; 6= Sesame; 7= Millet; 8= Rice

Section D: Harvesting and Post Harvest Handling

1. List the various rice post harvest handling processes that you undertake

Post harvest handling stage	Problems faced	Coping mechanism	Source of coping mechanism*
Harvesting			
Threshing			
Drying			
Milling			

* **Codes for source of coping mechanism: 1=own experience; 2=fellow farmer; 3=extension; 4=NGOs; 5=NARS; 6=farmer organization; 7=other (specify)**

2. Please indicate how you store your rice, problems associate with storage and how you cope with the problems.

Storage method*	Problems faced	Coping mechanism

***Storage method codes: 1=packed in sacs placed in the house; 2=granary; 3=silos; 4=spread on the floor in the house; 5=spread on tauplin in the house; 6=other, (specify)**

Section E: Rice Marketing

1. To whom do you sell your rice? **Wholesalers=1, Retailers=2, Consumers=3, others (specify)=4**

………..

2. How far is the nearest rice market (selling point) in km?

…………………………………………………………

3. How do you measure the amount of rice you sell?

……………………..……………………………………………

1=cup, 2=kilogram, 3=tin, 4=bag

4. What is the average price per kg of rice that you sell?

i) Paddy …………………………………..……………… ii) Milled rice

………………………………………………………………..

5. How do you transport your rice to the market? **1=foot, 2=bicycle, 3=motorcycle, 4=car, 5=other (specify)**

………

6. How much does it cost to transport rice to the market? (This should be by transport type)

Transport type	Transport cost

7. List the attributes that are considered in defining/determining the price of rice and how they influence price

Price defining attributes	How the influence price

8. What do you think about the rice price?
………………………………………………………………………………

9. Which factors hinder farmer involvement in the marketing of rice?
……………………………………….
………
…………………………………..…………

10. Which are the farmer organizations that are involved in the marketing of rice?

Organization	Marketing role of organization	Benefits to you/community

11. What is your role in the marketing of rice?
……………………………………………………………………………..

12. State any constraints that you face in rice marketing

Constraints	Rank	Coping mechanism*	Source of coping mechanism information**	Suggested solution***

***Coping mechanism refers to the way the farmer is dealing with the problem in question**
****Source of coping mechanism codes: 1=own experience; 2=fellow farmer; 3=extension; 4=NGOs; 5=NARS; 6=farmer organization; 7=other (specify)**
****Suggested solution refers to ways that the farmer thinks the problem in question could be dealt with but he/she is not using it.**

Key informant questionnaire

(Key informants include Extension agents, County Agriculture Commissioners, traders, millers, NGOs)

Section A: General information

Name of key informant ……………………………………………………………………………………………………
Name of organization represented …………………………………………………………………………………………………
Jurisdiction of the organization ……………………………………………………………………………………………………..
Position in the organization ……………………………………………………………………………………………………
Gender………………………………………………………………………………………………
Formal education……………………………………………………………………………..

Section B: Rice production

1. Please indicate type of your involvement in rice sector…………………………………………………………….………
2. Provide list of the major rice producing sub counties in the County (with evidence/documentation) ………………………………………………………………………………………………………

3. List the categories of all producers of rice in the County ……………………………………………………………… ………
4. Specify the number of households involved in rice production in the County/payam …………………………………………………………………………………………………… ………………………………………………..
5. State the total area (acres) under rice in the County/payam ……………………………………………………

6. State inputs used in rice production in the County/payam and their level of usage

Inputs	Input source (s)	Level of usage	Problems associated with access by farmer	Suggested solutions

7. Which rice varieties are produced in the County/payam and which ones are available in the market?

Rice varieties produced*	Rice varieties available in the market*	Comments

***Variety codes:1=Supa; 2=Kaiso; 3=Superica 1; 4=Nerica 4/Naric 3/Superica 2; 5=Nerica 1; 6=Nerica 10; 7=Nerica lowland; 8=Naric 1 ; 9=Naric 2 ; 10=Sindano; 11=IRAT, 12=TOX 5, 13=other traditional varieties (specify, e.g. Pakistani); 14=other (specify); 99=don’t know variety**

8. Where do farmers get financial support for rice production and marketing?
………
……………………….………………………………………………………………………………

9. When did rice farming start in this County/payam?
i) Upland ………………….……………………………
ii) Lowland ………………………………………..……………
iii) Irrigated …………………………………………………………….

10. Rice acreage and production in the years 2010-2014

Year	Ecology	Area under rice (acres/ feddans)	Production (kg)		Selling price per unit (specify)	
			Grain (kg)	Seeds (kg)	Grain	Seed
2014						
2013						
2012						
2011						
2010						

Note: 1 feddan =60mx70m

11. Where do the rice growers obtain technical skills and information from?
……………………………….

12. What are the methods/ technologies of rice production?

Rice production	Method/technology	Remark/comment
Planting		
Weeding		
Harvesting		
Threshing		
Drying		

13. State the most important problems faced in rice production

Problem	**Coping mechanism**	**Suggested solution**	**Remarks**

Section C: Rice post harvest handling

1. What problems are faced in rice processing?

Post harvest process	Problems	Coping mechanism	Suggested solution	Remark
Threshing				
Drying				
Storage				
Milling				
Transportation				

2. State any standards that exist with respect to quality, packaging, storage, transportation and post harvest handling
……..

Section D: Rice marketing

1. List the key players in rice marketing within the County/payam and their location

Key player	Role in rice marketing	Location	Remark

2. How do participants in rice marketing obtain information
………………………………………………………..

3. What is your rating of access to market information by farmers/key players in rice marketing in the district/payam?
…………………………………………………………..……………………………………………………

4. Suggest methods for improving access to market information
……………………………………………………

5. How does rice get to the market place?
………………………………………………………………………………………..

6. How do you rate the costs of transporting rice to the market?
……………………………………….…………...

7. How can the costs of transporting rice to the market be reduced?
…………………………………………………

8. Which methods are used for promoting rice marketing?
……………………….…………………………………….

9. How is the quality of rice maintained?
……………………………………………………………………………………………

10. Problems and opportunities associated with rice marketing

Problems	Coping mechanism	Suggested solution	Opportunities	Remarks

Section E: Research on Rice – Rice scientists
General expert opinion will be sought on all aspects of rice research

Indicate research undertaken on rice…………………………………………………………………………………

Who are involved in rice research
……………………………………………………………………………………………

Problems in rice research
………………………………………………………………………………………………………

Suggested solutions to the stated problems
…………………………………………………………………………………..

Section F: Overall assessment of the rice value chain

1. Indicate the key actors in the rice value chain
………………………………………………………………………………
2. Indicate how these existing actors are interlinked and describe problems associated with each of the links.
………
………………………………………………
………
………………………………………………
………
………………………………………………
3. Use the table below to indicate your assessment of t he rice value chain

Chain actor	**Strengths**	**Weaknesses and gaps**	**Opportunities / potential**	**Threats**

Focus group discussion checklist

Section A: Inputs supply

1. List the inputs used in rice production in order of importance
2. Sources of inputs used in rice production
3. Who offers you technical advice and information on inputs?
4. Where do you get financial support from?
5. Problems of input supply/ acquisition
6. How they are coping with the problems listed
7. Are there any ways they think the problems can be handled but are not being utilized by the community?
8. Why are you not using these solutions?

Section B: Rice production

1. List the categories of all producers of rice seed and grain (small, medium and large scale but must be defined by the farmers)
2. State the varieties of rice grown and when it was first grown in the area
3. Which variety is most preferred?
5. State why the variety named above is preferred
6. Which variety is least preferred?
7. State why the variety named above is least preferred?
8. What are the methods used in each stage of rice production?

Production	**Method used**		
	Method 1	**Method 2**	**Method 3**
First ploughing			
Second ploughing			
Harrowing			
Planting			
Weeding			
Harvesting			

9. State any arrangements (informal, partnerships, contracts) that exist for rice production

Arrangements	Criteria for qualifying as member	Activities under the arrangements	Benefits from the arrangements

10. Who offers rice growers technical skills and information in rice production?
……
…………………………………..

11. State the problems in rice production

Problems	Coping mechanism	Suggested solutions

Section C: Post harvest handling and processing

Please indicate how rice is handled after harvesting

Post harvest handling	Method used	Why that method	Problems encountered in using the method	Coping mechanism	Suggested solution
Threshing					
Drying					
Packaging					
Storage					
Milling					

Section D: Rice marketing by growers

1. Do you sell rice as seed, grain or both?
2. As seed, how do you package? And where do you sell?
3. How far is the nearest rice seed market (selling point)?
4. As grain, how do you package? And where do you sell?
5. How far is the nearest rice grain market (selling point)?
6. How do you measure the amount of fresh rice you sell? (Seed and grain)
7. How do you transport rice to the market?

Transport mode	Transport cost

8. Besides transport, State the types of costs incurred in rice marketing
9. How do you store rice during marketing?

10 How is the price of rice determined?

11. Which factors hinder farmer involvement in the marketing of rice?
12. Which are the farmer organizations that are involved in the marketing of rice?

Organization	Activities of organization	Membership requirement	Payment arrangement	Remark

13. What attributes are looked at as quality assurance and how are they handled to address quality?

Quality attributes (e.g. colour, aroma, impurities, etc.)	How attributes are addressed to maintain quality

14. What are the sources of market information for the growers?

Source	Cost associated with access	Payment arrangement	Problems associated with access	Coping mechanism	Suggested solution

15. What is the mode of payment (payment arrangement) for the rice grain? ……………………………………
……
……………………………………….

17. State any problems that you face in rice marketing

Problems	Coping mechanism	Suggested solution

Section E: Marketing of rice by traders

1. How many rice traders are here (by category i.e. retailers, wholesalers, brokers etc)

Trader category	Number
Retailers	
Wholesalers	
Brokers	

2. Who offers you business skills and market information?

3. What is the source of funds for purchasing the rice?

Initial source	Subsequent source	Problems faced	Coping mechanism	Suggested solution

4. Explain the cause of rice shortage

Time of the year shortage experienced (month of the year)	Cause of shortage	Coping mechanism

5. State any trade associations that exist in you area of operation

Association	Activities of association	Membership requirement	Benefits from association	Remark

Rice variety sold	Source of the variety	Mode of transport to market place	Preferred variety in the market	Reasons for the preferred variety

6. Indicate the rice variety sold, source, mode of transportation to the market

8. What problems are associated with marketing of rice?

Problem	Coping mechanism	Suggested solution

Evaluating the cooking and eating quality and preferred rice agro-morphological traits for upland and lowland rice cultivars

A rice cooking and eating quality exercise was conducted to capture stakeholders' general views on rice cooking and taste qualities. In this exercise, test materials included imported rice varieties (Basmati, Pakistan, and China) and locally cultivated rice cultivars (NERICA 4 and NERICA 1) which were used as controls. The materials were acquired from the local market where imported rice was observed to be slightly more expensive than the locally produced rice.

The test materials were treated equally during the cooking process and were branded with letters to avoid any bias. Thereafter, stakeholders were able to score the cultivars on a scale of 1 (most preferred) to 5 (least preferred) for cooking and eating quality giving reasons for like or dislike. Furthermore, a participatory variety selection (PVS) was conducted for upland and lowland cultivars as a means to identify farmers' needs in a cultivar and to expose stakeholders to new rice cultivars. Test materials considered included both landraces and new introductions in the rice breeding programme in South Sudan. Stakeholders were able to score the cultivars based on desirable agro-morphological attributes following a scale of 1 (most preferred) to 5 (least preferred). The scores were then tallied, followed by matrix ranking of the most preferred stakeholders' agro-morphological and cooking quality traits in a rice cultivar.

Table i: Matrix ranking of stakeholder variety preferences for cooking and eating quality attributes during a focus group discussion

	Individual rank					Rank	Overall	Preference	
Variety	1	2	3	4	5	index	rank	Like	Dislike
A (Pakistan)									
B (Basmati)									
C (China)									
D (NERICA 1)									
E (NERICA 4)									

Table ii: Matrix ranking of stakeholder preferences for agro-morphological traits in lowland rice cultivars during a focus group discussion

	Individual rank			Rank	Overall	Preferences	
Variety	1	2	3	index	rank	Like	Dislike
A (326104)							
B (NERICA-L-19)							
C (Supa 1052)							
D (K-85)							
E (1189)							
F (Kumboka)							
G (Supa-TZ)							
H (NERICA-L-6)							
I (TXD-306)							
J (Wita 9)							

Table iii: Matrix ranking of stakeholder preferences for agro-morphological traits in upland rice cultivars during a focus group discussion

Variety	Individual rank			Rank index	Overall rank	Preference	
	1	2	3			Like	Dislike
A (P5 H6)							
B (ART2-4L3P1-2-1)							
C (ART3 -8L6P3-2-3-B)							
D (NERICA 1)							
E (ART3-7L9P8-3-5-B-B-2)							
F (ART3 -7L3P3-B-B-2)							
G (SCRIDO 06-2-4-3-4-5)							
H (ART25-3-29-2-B)							
I (NERICA 4)							
J (ART12-L2P2-20-3-1-1)							