IDENTIFICATION OF GENOMIC LOCI UNDERLYING NUTRITIONAL QUALITY TRAITS IN COMMON BEAN (*Phaseolus vulgaris* L.) AND PARTICIPATORY EVALUATION AND SELECTION OF NUTRITIONALLY SUPERIOR COMMON BEAN GENOTYPES WITH FARMERS

By

Dennis Ndahura Katuuramu

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Breeding, Genetics and Biotechnology- Crop and Soil Sciences-Doctor of Philosophy

2017

ABSTRACT

IDENTIFICATION OF GENOMIC LOCI UNDERLYING NUTRITIONAL QUALITY TRAITS IN COMMON BEAN (*Phaseolus vulgaris* L.) AND PARTICIPATORY EVALUATION AND SELECTION OF NUTRITIONALLY SUPERIOR BEAN GENOTYPES WITH FARMERS

By

Dennis Ndahura Katuuramu

Micronutrient malnutrition, particularly iron and zinc affects millions of people around the world. Biofortification (nutrient enhancement in staple crops) has a potential to address micronutrient malnutrition especially for vegetarians and people who heavily rely on plant-based diets. Common bean (*Phaseolus vulgaris* L.) is the most important food legume crop grown worldwide. It is also an important component of production systems and a major source of protein, fiber, and minerals to millions of people. However, the full genetic potential of common bean to supply iron and zinc to humans is not well understood. Also, genotype x environment interactions and growers' priority traits for biofortified crops are not adequately studied. Three research objectives were designed to address these knowledge gaps. Chapter 1 details the efforts made to uncover genomic loci underlying traits of nutritional importance (protein, zinc, calcium, and iron bioavailability) using genome-wide association study (GWAS). The GWAS panel was evaluated for two seasons (2012 and 2013) under field conditions in Michigan. The GWAS experiment identified several SNP markers, candidate genes, and superior germplasm that can be deployed for marker assisted breeding and selection. Phenotypic screening of the GWAS panel revealed large variability for mineral concentration (up-to 3-fold variation) and iron bioavailability (over 5-fold variation). Candidate genes and marker-trait associations for seed zinc, calcium, and iron bioavailability were discovered on chromosomes Pv07, Pv04, and Pv11 respectively. In chapter 2 a subset of 15 ADP

genotypes selected for micronutrient composition, cooking time, and iron bioavailability were evaluated on farmers' fields at nine locations across three agro-ecological zones in Uganda for two years (2015 and 2016). A GGE biplot analysis was used to investigate genotype by environment interactions for traits of interest. Seed yield was largely controlled by location (21.5 %) and the interaction between location and season (48.6 %). Cooking time was mostly controlled by genotype (40.6 %). Seed Fe concentration was largely controlled by genotype (27 %) and location (15 %). Seed Zn concentration was also mostly controlled by genotype and location effects at 23.1 and 25.5 % respectively. Chapter 3 describes a survey of farmers' priority traits for biofortified beans and sensory analyses of five farmer selected genotypes using participatory variety selection. The most important bean variety attributes according to growers and consumers were early maturing, high yielding, fast cooking, and flavorful. Sensory panelists preferred flavorful beans with soft texture. The GWAS, G x E, and on-farm participatory variety selection and sensory evaluation research objectives generated novel germplasm for high seed Fe and Zn concentration, fast cooking, and candidate SNPs and genes that can be used to improve common bean for nutritional quality traits.

To My Beloved Parents: Mom and Dad, I am Eternally Grateful for Everything!!

ACKNOWLEDGMENTS

I am grateful to my major Professor Dr. Karen A. Cichy for her kindness and support during my PhD program at MSU. I appreciate her counsel especially during the early years of my PhD program when I had to design and benchmark my research experiments. She also supported and encouraged me to take on challenging experiments for which I am thankful.

I am equally indebted to Dr. James D. Kelly for serving on my graduate committee. I enjoyed learning about the foundational knowledge in plant breeding and genetics from his graduate course. Also, the plant breeding discussions I had with Dr. Kelly in the common bean fields both in Michigan and Uganda helped expand my understanding of dry bean breeding and genetics.

I am also thankful to Drs. Muraleedharan G. Nair and Leslie D. Bourquin for their willingness to serve on my graduate committee. Their presence on the committee helped expand my understanding of certain aspects of common bean chemistry and human nutrition.

I would like to thank both past and current members of the Cichy and Kelly bean laboratories for their support while I conducted my research studies.

I am also grateful to the Almighty God through whom all things are possible.

TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES xii

GENERAL INTRODUCTION 1
GENERAL INTRODUCTION 2
Common bean production and consumption 2
Global status of iron and zinc malnutrition and impact of biofortified crops 2
Mineral composition and genetic variability in common bean 4
Iron bioavailability in common bean 5
Breeding for iron and zinc concentration 6
Dissertation outline 8
LITERATURE CITED 10

CHAPTER 1 18
GENOME-WIDE ASSOCIATION ANALYSIS OF NUTRITIONAL COMPOSITION RELATED TRAITS AND IRON BIOAVAILABILITY IN COOKED COMMON BEAN (*Phaseolus vulgaris* L.) SEEDS 18
Genome-wide association analysis of nutritional composition related traits and iron bioavailability in cooked common bean (*Phaseolus vulgaris* L.) seeds 19
Abbreviations 19
Abstract 20
Introduction 21
Materials and Methods 26
Field site characteristics 26
Germplasm and field plot design 26
Phenotyping of nutritional quality traits 27
Preparation and cooking of seed for nutritional analysis 27
Cooked seed protein measurement 27
Quantification of cooked seed zinc, iron, potassium, calcium, and phosphorus concentrations 27
Quantification of iron bioavailability 28
Determination of seed phytic acid 29
Genotyping-by-sequencing of the Andean diversity panel 29
DNA isolation and quantification 29
GBS library construction and sequencing 30
Sequence processing, alignment, SNP genotyping 30
Linkage disequilibrium, population structure, and kinship analyses 31
Phenotypic data analysis 32
Genome-wide marker-trait association analysis 32
Candidate gene identification 34
Results 35
Phenotypic trait summary statistics 35

Phenotypic correlations 36
Population structure and genome-wide marker-trait associations 37
Seed protein concentration 38
Seed zinc concentration 38
Seed calcium concentration 39
Iron bioavailability 39
Discussion 40
Marker-trait associations 42
Favorable marker allelic classes in the ADP germplasm 43
Candidate genes associated with the significant SNPs for the evaluated traits 44
Conclusion 47
Acknowledgements 47
LITERATURE CITED 60

CHAPTER 2 70
EVALUATION OF GENOTYPE BY ENVIRONMENT INTERACTIONS FOR AGRONOMIC, COOKING TIME, AND NUTRITIONAL QUALITY TRAITS IN COMMON BEAN ACCESSIONS GROWN ON-FARM IN UGANDA 70
Evaluation of genotype by environment interactions for agronomic, cooking time, and nutritional quality traits in common bean accessions grown on-farm in Uganda 71
Abstract 71
Introduction 72
Materials and Methods 75
Field study sites 75
Common bean germplasm 76
Experimental design 77
Phenotyping for bean foliar diseases and agronomic traits 77
Phenotyping for cooking time and mineral nutrient composition 78
Statistical analyses 79
Results 80
Soil chemistry characteristics 80
ANOVA, summary statistics, heritability, and trait correlations 82
Agronomic traits 82
End use quality 82
Seed nutrient density 83
Disease scores 83
Correlations among traits 84
Evaluation of genotype and environment performances 85
Agronomic traits 85
End use quality 86
Seed nutrient density 87
Disease scores 89
Polygon (“which-won-where”) view of the GGE biplots 91
Yield, cooking time, and seed Fe and Zn concentration 91
Seed Ca, K, and P concentrations 92
Genotype mean performance vs. stability GGE biplots 92

Yield performance 93
Cooking time and seed Fe and Zn concentration 93
Genotype rankings with reference to the ideal genotype 94
Yield and cooking time 94
Seed Fe and Zn concentration 95
Discussion 95
Conclusion 99
LITERATURE CITED 140

CHAPTER 3 145
IDENTIFICATION OF FARMERS' PRIORITIES FOR HIGH MINERAL AND FAST COOKING DRY BEANS (*Phaseolus vulgaris* L.) THROUGH PARTICIPATORY VARIETY SELECTION AND SENSORY EVALUATION IN UGANDA 145
Identification of farmers' priorities for high mineral and fast cooking dry beans (*Phaseolus vulgaris* L.) through participatory variety selection and sensory evaluation in Uganda 146
Abstract 146
Introduction 147
Materials and Methods 151
Farmer group selection and collection of socio-economic data 151
Plant materials and farmer selection of top four genotypes 151
Selection of consumer sensory panelists 152
Preparation of the bean samples for sensory evaluation 153
Consumer sensory evaluations 153
Statistical data analyses 154
Results and Discussion 155
Socio-economic characteristics of the study respondents 155
Bean production and consumption 156
Farmers' characteristics of the studied common bean genotypes 158
Bean cooking time at the sensory evaluation locations 159
Genotype effects on sensory attributes at the three central locations 159
Additional crops grown by the surveyed farmers 161
Conclusion 162
LITERATURE CITED 170

GENERAL CONCLUSIONS AND FUTURE RESEARCH 175
Recommendations for future research 178

APPENDICES 179
Appendix A: MSU IRB training certificate for Dennis N. Katuuramu 180
Appendix B: MSU IRB approval letter for the research project 181
Appendix C: Informed consent form to be completed by the respondents 182
Appendix D: Survey instrument for demographic, bean production and consumption characteristics of the respondents in the high mineral dry bean evaluation project in Uganda 184
Appendix E: Survey instrument for sensory evaluation of the high mineral dry bean acceptability study in Uganda 188

LIST OF TABLES

Table 1.1: Phenotypic summary statistics of the nutritional traits in cooked seed samples for all the 206 common bean genotypes grown in Michigan, USA for 2012 and 2013 field seasons. ... 48

Table 1.2: ANOVA showing mean squares and percentage of total variance explained for seed protein, zinc, iron, and iron bioavailability of 206 common bean genotypes evaluated for two field seasons at Montcalm research farm in Michigan, USA. 49

Table 1.3: ANOVA showing mean squares and percentage of total variance explained for seed potassium, calcium, phosphorus, and phytic acid of 206 common bean genotypes evaluated for two field seasons at Montcalm research farm in Michigan, USA. 50

Table 1.4: Pearson correlation coefficients among nutritional traits for all the 206 common bean genotypes grown in Michigan, USA for the 2012 and 2013 field seasons..... 51

Table 1.5: Details of the top five loci that were significantly associated with the nutritional traits identified using MLM-based genome-wide association study across the 206 common bean genotypes grown in Michigan, USA for the 2012 and 2013 field seasons..... 52

Table 2.1: Description of the 15 genotypes evaluated in the study in Uganda……101

Table 2.2: Description of the local checks evaluated along with the ADP test genotypes in the study in Uganda 102

Table 2.3: Description of the nine locations used for the genotype x environment study in Uganda 103

Table 2.4: Soil chemical composition analysis for the nine study locations over the two years in Uganda 104

Table 2. 5: ANOVA showing mean squares and percentage of total variance explained for yield, 100-seed weight, and cooking time of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda..... 105

Table 2.6: ANOVA showing mean squares and percentage of total variance explained for seed iron, zinc, and calcium concentrations of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda..... 106

Table 2.7: ANOVA showing mean squares and percentage of total variance explained for seed potassium, phosphorus, and magnesium concentrations of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda..... 107

Table 2.8: ANOVA showing mean squares and percentage of total variance explained for the bean foliar diseases angular leaf spot, common bacterial blight, and common bean rust among 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda 108

Table 2.9: ANOVA showing mean squares and percentage of total variance explained for the bean common mosaic virus and black root among 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda 109

Table 2.10: Descriptive summary statistics and broad sense heritability estimates for agronomic, cooking time, nutrient composition traits, and response to biotic stresses of 16 common bean genotypes grown across nine locations for two years in Uganda 110

Table 2.11: Pairwise correlation coefficients among traits averaged over the nine locations and two field seasons 111

Table 2.12: Genotype means for the observed traits of 16 common bean genotypes evaluated across nine field sites for two years in Uganda..... 112

Table 2.13: Mean performance of the local check common bean varieties for the observed traits evaluated for two years in Uganda..... 113

Table 2.14: Environmental means for the measured traits across 16 common bean genotypes evaluated in nine field sites for two years in Uganda 114

Table 3.1: Socio-economic characteristics of bean growers in Hoima, Kamuli, and Rakai districts of Uganda 163

Table 3.2: Farmers' choice of bean seed types for production in Hoima, Kamuli, and Rakai districts in Uganda 163

Table 3.3: Farmers' choice of bean seed types for consumption in Hoima, Kamuli, and Rakai districts in Uganda 164

Table 3.4: Reasons for farmers' choice of common bean seed types to grow in Hoima, Kamuli, and Rakai districts in Uganda 164

Table 3.5: Reasons for farmers' choice of common bean seed types to consume in Hoima, Kamuli, and Rakai districts in Uganda 165

Table 3.6: Bean genotype characteristics emphasized by growers during flowering, harvesting, and at threshing as their top five choices in Uganda 166

Table 3.7: Cooking time of the five genotypes evaluated in the sensory study at the three central locations in Hoima, Kamuli, and Rakai districts of Uganda..... 167

Table 3.8: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Hoima district of Uganda..... 167

Table 3.9: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Kamuli district of Uganda..... 168

Table 3.10: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Rakai district of Uganda 168

Table 3.11: List of other crops grown by the surveyed dry bean farmers in the districts of Hoima, Kamuli, and Rakai in Uganda 169

LIST OF FIGURES

Figure 1.1: Histogram showing the distribution of seed protein, zinc, iron, and iron bioavailability in the cooked bean samples 54

Figure 1.2: Histogram showing the distribution of seed potassium, calcium, phosphorus, and phytic acid concentration in the cooked bean samples 55

Figure 1.3: Manhattan plots depicting genome-wide marker-trait association for seed protein concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated 56

Figure 1.4: Manhattan plots depicting genome-wide marker-trait association for seed zinc concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated 57

Figure 1.5: Manhattan plots depicting genome-wide marker-trait association for seed calcium concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated 58

Figure 1.6: Manhattan plots depicting genome-wide marker-trait association for seed FeBIO for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated 59

Figure. 2.1: Map of Uganda showing the study districts of Hoima, Kamuli, Masaka, and Rakai for the multi-location on-farm evaluation of the 16 common bean genotypes for two field seasons of 2015 and 2016115

Figure. 2.2: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 116

Figure 2.3: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 117

Figure 2.4: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two

years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 118

Figure 2.5: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 119

Figure 2.6: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 120

Figure 2.7: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 121

Figure 2.8: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 122

Figure 2.9: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 123

Figure 2.10: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 124

Figure 2.11: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 125

Figure 2.12: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two

years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 126

Figure 2.13: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 127

Figure 2.14: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 128

Figure 2.15: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 129

Figure 2.16: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 130

Figure 2.17: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 131

Figure 2.18: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 132

Figure 2.19: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate

(AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 133

Figure 2.20: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 134

Figure 2.21: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 135

Figure 2.22: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 136

Figure 2.23: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 137

Figure 2.24: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 138

Figure 2.25: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype

is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively. 139

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Common bean production and consumption

Common bean (*Phaseolus vulgaris* L.) is the most widely grown pulse crop in the world (Asfaw et al., 2009; Akibode and Maredia, 2011). Common bean grown and consumed around the world belongs to two gene pools (Middle American and Andean). The two gene pools can be distinguished using morphological, physiological, and molecular characteristics (Tohme et al., 1996). The Andean seed types have larger leaves, longer internodes on the main stem, larger seeds and mostly white flowers while the Middle American gene pool contains genotypes with smaller seed sizes, shorter internodes, and mostly colored flowers (Singh et al., 1991). At the molecular level, the two gene pools can be distinguished by the isoforms/types of phaseolin (which is a seed storage protein). The Middle American gene pool contain the S phaseolin type while the Andean beans contain the T phaseolin isoform (Kami and Gepts, 1994).

Global production of common bean exceeds 23 million metric tonnes, out of which, 7 million tonnes are produced in Africa and Latin America (Akibode and Maredia, 2011) with 60% of the world production belonging to the Middle American gene pool (Beebe et al., 2000; Miklas and Singh, 2007). Dry beans are a major source of protein, dietary fiber and minerals for consumers in sub-Saharan Africa (Broughton et al., 2003; Asfaw et al., 2009; Akibode and Maredia, 2011). Dry beans are a rich source of micronutrients especially iron and zinc (Blair, 2013), this coupled with their wide consumption as staples in developing countries, have led to their selection as targets for mineral biofortification (Bouis and Saltzman, 2017; Petry et al, 2015; Bouis and Welch, 2010; Pfeiffer and McClafferty, 2007).

Global status of iron and zinc malnutrition and impact of biofortified crops

Micronutrient malnutrition, also called “hidden hunger” (Kennedy et al., 2003), especially for iron (Fe) and zinc (Zn) afflicts about two billion people around the world and most of the

affected populations are resource-limited people living in developing countries (Nestel et al., 2006; Hirschi, 2009). The most vulnerable segments of the population for Fe and Zn deficiencies in developing countries are women, especially those that are pregnant or nursing, and infants and pre-school children (Gibson, 2006; Zimmermann and Hurrell, 2007). Iron deficiency in humans can result in mental impairment, high morbidity rates, and physical incapacitation (Boccio and Iyengar, 2003; Caballero, 2002). Zinc deficiency results in child and infant stunting, impaired immunity, and mortality (Bouis, 2003). Nearly, 500,000 children under five years die annually due to Fe and Zn malnutrition (Black et al., 2008).

Some of the approaches to combat micronutrient malnutrition are dietary diversification, mineral and vitamin supplementation, and food fortification. These methods have however had limited success due to the high implementation/ delivery costs and lack of sustainability (Pinstrup-Andersen, 2002; Hirschi, 2009). Moreover, resource limited populations rely on less diverse food diets that usually contain plant based staples for their calories, minerals, and protein (Bouis, 2000; Welch and Graham, 2000). Therefore, a more effective and sustainable approach to address micronutrient malnutrition in the global south is to increase amounts of bioavailable micronutrients in the edible portions of plants through conventional and molecular plant breeding- a process called biofortification (Genc et al., 2005; Pfeiffer and McClafferty, 2007). Increasing Fe and Zn in dry bean is possible (da Rosa et al., 2010). Other successful examples of crop biofortification include quality protein maize (QPM), which has high levels of balanced dietary protein (Atlin et al., 2011), cassava rich in beta-carotene (a precursor for vitamin A) (Akinwale et al., 2010; La Frano et al., 2013), and orange-fleshed sweet potatoes that are rich in beta-carotene (Hotz et al., 2012; Low et al., 2007).

The recommended dietary allowance for Fe in human beings varies from 8 to 18 mg per day depending on the gender, age, and stage of growth (Nishimuta et al., 2012). Men typically require less Fe with levels of 8 mg per day considered sufficient while actively growing children and teenagers require 15 mg per day (Domellof et al., 2014). Women require up to 18 mg of iron per day for their normal body functioning. The tolerable upper limit of Fe per day is up to 45 mg per day (Nishimuta et al., 2012). Typically, high amounts of Fe levels cannot be absorbed from foods due to low bioavailability resulting from presence of Fe absorption inhibitors like phytates, tannins, and some polyphenolic compounds in foods. Dry bean has the potential to supply up to 8 mg from a single cup serving thus considered a high Fe food. The development of bean genotypes high in Fe and also low in phytate and polyphenolic compounds would be helpful to address Fe malnutrition (Petry et al., 2015).

Mineral composition and genetic variability in common bean

Screening for micronutrient concentration in dry bean has revealed a wide range of variability in seed Fe and Zn concentrations (Beebe et al., 2000; Nunez-Gonzalez et al., 2002; Wissuwa, 2005). Graham et al., (1999) and Beebe et al., (2000) reported a wide range of variability in levels of seed Fe in dry bean with amounts ranging from 30 to 150 µg g^{-1}. Blair et al., (2009) working on an inter-gene pool recombinant inbred line population highlighted the quantitative nature of Fe and Zn inheritance in common beans with 40-90 µg g^{-1} and 17-40 µg g^{-1} for seed Fe and Zn contents, respectively. Islam et al., (2002) working on a core collection of 1,072 common bean genotypes found seed Fe to range from 35-92 µg g^{-1} while seed Zn varied from 21-59 µg g^{-1}. This indicates that common bean has sufficient variability that can be selected upon in conventional plant breeding or biofortification programs.

Iron bioavailability in common bean

Beyond making significant increases in iron concentration in the edible portions of staple foods, it is equally important to determine how much of those nutrients are actually bioavailable (Hirschi, 2008). The bioavailability of a nutrient is defined as the proportion of the ingested micronutrient that is absorbed and used for normal body biological functions (Cockell, 2007; Fairweather-Tait et al., 2007). Bioavailability will depend on the dietary factors (food matrix) and characteristics of the individual. Phytic acid and some classes of polyphenols present in foods can be major inhibitors of iron (Hurrell and Egli, 2010) absorption from plant based diets since they chelate the minerals making them unavailable for human absorption when in the human gut.

Mineral bioavailability can be assessed through various methods like algorithmic approximations, *in vitro* digestion using Caco-2 intestinal cells, poultry studies, and human studies (Cockell, 2007; Glahn et al., 1998; Tako et al., 2010). The algorithmic method is the least suited to predict the effects of new circumstances, like the nutritional impact of a new biofortified crop. Decisions on the choice of other methods are typically based on experimental costs, nature of required responses (short vs. long-term), and differences in mineral absorption between laboratory animals and humans (Cockell, 2007). Quantifying mineral bioavailability via human and animal studies is expensive and for plant breeding purposes large screening of genotypes for this trait can be reasonably achieved using *in vitro* assays (Wienk et al., 1999).

The current established *in vitro* screening method involves simulated gastric and intestinal digestion of food coupled with measurement of iron uptake by the human intestinal epithelial cells or the Caco-2 cell line (Glahn et al., 1998). The caco-2 cell line has characteristics of the small intestine epithelial cells, which is believed to be the primary site for iron absorption in the human gastrointestinal tract. The caco-2 cells have shown a wide range of morphological and functional characteristics of intestinal epithelia in regards to the uptake of iron and other nutrients, making it

a powerful system for quantifying iron uptake (Glahn et al., 1998; Jovani et al., 2001). These characteristics include: 1) Caco-2 cells reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron through the apical Fe uptake pathway and tightly regulate ferritin synthesis and trans-epithelial Fe transport within a narrow margin of intracellular Fe concentration (Tapia et al., 1996), 2)Transport of Fe in the Caco-2 cell line system responds to the Fe status of the cell, where Fe-deficient cells show increased transport while Fe-loaded cells exhibit decreased transport into the baso-lateral side of the cells (Alvarez-Hernandez et al., 1991), 3) Factors that inhibit Fe availability like phytate and certain polyphenols or promoters of Fe availability (vitamin C) have similar effects on Fe uptake in the Caco-2 cells as they do in human or animal subjects (Glahn and van Campen, 1997; Garcia-Casal et al., 2000). Also, a comparison study using both human subjects (by measuring Fe absorbed in the body from food) and the ferritin formation from the Caco-2 cell system concluded that estimation of bioavailable iron from foods using human subjects and Caco-2 cells was similar (Yun et al., 2004). Information on genetic studies for iron bioavailability in dry beans is lacking. The Caco-2 cell culture methodology was used to determine the variability for seed iron bioavailability present in common bean genotypes.

Breeding for iron and zinc concentration

Studies on the inheritance of Fe and Zn content have been conducted in common bean using biparental populations (Cichy et al., 2009; Blair et al., 2009, 2010). These studies demonstrated that inheritance of Fe and Zn was quantitative in nature. Approximately 38 to 40 quantitative trait loci (QTLs) associated with Fe and Zn accumulation, respectively, have reported. These QTLs explained 15-40 % variability in Fe and Zn accumulation or mineral content in seeds.

Genome-wide association studies (GWAS) involve the examination of genetic variants among many genotypes or cultivars to determine if the variants are associated with the traits of interest. GWAS focuses on associations between SNPs and crop phenotypic traits (Korte and

Farlow, 2013). The method was first used in humans to study genetic architecture of human diseases (McCarthy et al., 2008; Witte, 2010) but it has now been used in many plant species to decipher the genetic control of a number of quantitative traits in common bean (Kamfwa et al., 2015), Arabidopsis (Atwell et al., 2010), in maize nested association mapping populations (Poland et al., 2011; Kump et al., 2011), and rice (Huang et al., 2010). Genome wide association mapping requires assembly of large diversity panels and generation of thousands of SNP markers (Hamblin et al., 2011; Ingvarrson and Street, 2010). The method offers numerous advantages. First, existing materials like landraces, breeding lines, and varieties can be assembled and used without the need to make crosses and multiple generation of selfing like in the development of recombinant inbred lines or double haploids through crosses and selfing (Oraguzie et al., 2007). Second, large numbers of alleles per locus can be mapped instead of the two alleles segregating in bi-parental populations (Zhu et al., 2008). Third, GWAS utilizes historical meiotic events (recombinations) that have occurred over a large number of generations, which in turn improves the resolution of the uncovered marker-trait associations (Aranzana et al., 2005).

With the current state of the art sequencing platforms (Thudi et al., 2012), common bean adapted genotyping by sequencing (Schroder et al., 2016), and the chromosome-scale common bean genome (Schmutz et al., 2014) we can generate millions of single nucleotide polymorphic (SNP) markers that can be used to detect QTLs conditioning mineral uptake, transport, and accumulation in common bean seeds.

Nutritional quality traits especially seed Fe and Zn concentration are complex in nature (i.e. controlled by multiple loci) and are affected by the environment (Blair et al., 2010). It is therefore salient to evaluate promising high mineral common bean genotypes for the quality traits in multiple locations to determine magnitudes of genotype by environment (G x E) interactions.

The G x E studies are also useful in the identification of test locations for germplasm evaluations, variety selection, and release (Annicchiarico et al., 2005). When nutrient dense dry beans are identified it is important to evaluate them for yield stability and reactions to biotic and abiotic constraints. In addition to yield performance, another avenue for determining whether growers will adopt biofortified crops is to engage them in field genotype evaluation using participatory variety selection approach (Gyawali et al., 2007). Consumer sensory evaluations using subjective hedonic scales have also been widely used to gauge farmer's interests in new biofortified crop varieties (Birol et al., 2015; Chowdhury et al., 2011). Currently a large gap exists between the yield potential of common bean varieties vs. that observed in farmers' fields in Uganda (Awio et al., 2017). Yields on-farm can be as low as 400 kg ha^{-1} when actual yield potential is 2000 kg ha^{-1} (Awio et al., 2017). These differences in observed bean performances can be due to poor crop management during the growing season, limited use of agricultural inputs, poor soil fertility, changes in weather patterns, and foliar disease pressures (Kiwuka et al., 2012; Okii et al., 2014). As result, breeding for yield and disease resistance has been the major focus for Uganda dry bean breeding programs. With high levels Fe and Zn malnutrition in the Ugandan population, high Fe beans have been targeted for inclusion into the national bean breeding objectives. One of the most important activities in nutrition breeding is characterization of bean germplasm for high seed Fe and Zn concentration and then assess genotype x environment interaction (Ortiz-Monasterio et al., 2007). Soil micronutrient status varies across marginal farming lands in sub-Saharan Africa including Uganda. Therefore, biofortified beans need to be evaluated across multiple and different agro-ecological zones to assess G x E for seed Fe and Zn concentrations in new bean germplasm.

Dissertation outline

It is against the above background that three interconnected and complementary research studies were carried out to investigate the genetic architecture of nutritional quality traits,

determine the magnitude of genotype by environment interaction for quality phenotypes, and gain insights into the drivers of farmer's adoption of biofortified dry beans. The dissertation outline is as shown below:

Chapter 1 is a Genome-wide association analysis of nutritional composition related traits and iron bioavailability in cooked common bean (*Phaseolus vulgaris* L.) seeds. The study was conducted on genotypes grown for two field seasons (2012 and 2013) in Michigan, USA.

Chapter 2 is a study on the evaluation of genotype x environment interactions for agronomic, cooking time, and nutritional quality traits in common bean (*Phaseolus vulgaris* L.) accessions grown on-farm in Uganda. The study was conducted at nine locations for two field seasons (2015 and 2016) in Uganda.

Chapter 3 is focused on the identification of farmers' priorities for high mineral and fast cooking dry beans (*Phaseolus vulgaris* L.) through participatory variety selection and consumer sensory evaluation in Uganda.

The general conclusion provides a summary of results for the GWAS, G x E, and participatory variety evaluation and consumer sensory evaluation experiments.

LITERATURE CITED

LITERATURE CITED

Akibode, C.S., Maredia, M. 2011. Global and regional trends in production, trade and consumption of food legume crops. Report submitted to the Standing Panel on Impact Assessment (SPIA) of the CGIAR Science Council, FAO, Rome.

Akinwale, M.G., Aladesanwa, R.G., Akinyele, B.O., Dixon, A.G.O., Odiyi, A.C. 2010. Inheritance of β-carotene in cassava (*Manihot esculenta* crantza). Intern J Genet Mol Biol 2: 198-201.

Alvarez-Hernandez, X., Nichols, G.M., Glass, J. 1991. Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. Biochim. Biopys. Acta. 1070:205-208.

Annicchiarico, P., Bellah, F., Chiari, T., 2005. Defining sub-regions and estimating the benefits of for a specific-adaptation strategy by breeding programs: A case study. Crop Sci. 45:1741-1749.

Aranzana, M.J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., Lister, C., Molitor, J., Shindo, C., Tang, C., et al., 2005. Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. PLoS Genet 1: e60.

Asfaw, A., Blair, M.W., Almekinders, C., 2009. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the east African highlands. Theor Appl Genet 120: 1-12.

Atlin, G.N., Palacios, N., Babu, R., Das, R., Twumasi-Afriyie, S., Friesen, D.K., De Groote, H., Vivek, B., Pixley, K.V. 2011. Quality Protein Maize: Progress and Prospects. In: Janick J, editor, Plant Breed Rev 34: 83-130.

Atwell, S., Huang, Y.S., Vilhajamson, B.J., Willens, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A.M. et al., 2010. Genome-wide association study of 107 phenotypes in a common set of *Arabidopsis thaliana* inbred lines. Nature 465: 627-631.

Awio, B., Mukankusi, C.M., Nkalubo, S.T., Gibson, P., Malinga, M.G, Rubaihayo, P.R., Edema, R., 2017. Variety x Environment interaction of diseases and yield in selected common bean varieties. Agron. J. 109:2450-2462.

Beebe, S., Skroch, P.W., Tohme, J., Duque, M.C., Pedraza, F., Nienhuis, J. 2000. Structure and genetic diversity among common bean landraces of middle American origin based on correspondence analysis of RAPDS. J. Crop Sci 40: 264-273.

Birol, E., Meenakshi, J.V., Oparinde, A., Perez, S., Tomlins, K., 2015. Developing country consumers' acceptance of biofortified foods: a synthesis. Food Secur. 7:555-568.

Black, R.E., Allen, L.A., Bhutta, Z.A., Caulifield, L.E., de Onis, M., Ezzati, M., Mathers, C., Rivera, J., 2008. Maternal and child under-nutrition: Global and regional exposures and health consequences. Lancet 371: 243-260.

Blair, M.W., 2013. Mineral biofortification strategies for food staples: The example of common bean. J Agric Food Chem 61: 8287-8294.

Blair, M.W., Astudillo, C., Renfigo, J., Beebe, S., Graham, R. 2010. QTL analyses for seed iron and zinc concentration and content in an intra-genepool population of Andean common beans (*Phaseolus vulgaris* L). Theor Appl Genet 122: 511-521.

Blair, M.W., Asudillo, C., Grusak, M.A., Graham, R., Beebe, S., 2009. Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L). Mol Breed 23: 197-207.

Boccio, J.R., Iyengar, V., 2003. Iron deficiency: Causes, consequences, and strategies to overcome this nutritional problem. Biol Trace Elem Res 94:1-32.

Bouis, H.E., 2000. Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. Nutr 16: 701-704.

Bouis, H.E., 2003. Micronutrient fortification of plants through plant breeding: Can it improve nutrition in man at a low cost? Proc Nutr Soc 62: 403-441.

Bouis, H.E., Saltzman, A., 2017. Improving nutrition through biofortication: A review of evidence from HarvestPlus, 2003 through 2016. Global Food Security 12:49-58.

Bouis, H.E., Welch, R.M. 2010. Biofortification- A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50: S20-S32.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J. 2003. Beans (*Phaseolus* spp.) - model food legumes. Plant Soil 252:55-128.

Caballero, B., 2002. Global patterns of child health: The role of nutrition. Ann Nutr Metab 46: S3-S7.

Chowdhury, S., Meenakshi, J.V., Tomlins, K., Owori, C., 2011. Are consumers in developing countries willing to pay more for micronutrient-dense biofortified foods? Evidence from a field experiment in Uganda. Am. J. Agric. Econ. 93:83-97.

Cichy, K.A., Caldas, G.V., Snapp, S.S., Blair, M.W., 2009. QTL analysis of seed iron, zinc, and phosphorus levels in an Andean bean population. Crop Sci 49: 1742-1750.

Cockell, K.A., 2007. An overview of methods for assessment of iron availability from foods

nutritionally enhanced through biotechnology. J. AOAC Int. 90:1480-1491.

da Rosa, S.S., Ribeiro, N.D., Jost, E., Reiniger, L.R.S., Rosa, D.P., Cerutti T., Possobom, M.T.D.F., 2010. Potential for increasing the zinc content in common bean using genetic improvement. Euphytica 175: 207-213.

Domellof, M., Braegger, C., Campoy, C., Colomb, V., Decsi, T., Fewtrell, M., Hojsak, I., Mihatsch, W., Molgard, C., Shamir, R., Turck, D., van Goudoever, J., 2014. Iron requirements of infants and toddlers. J. Pediatric Gastro. Nutr. 58:119-129

Fairweather-Tait S., Phillips, I., Wortley, G., Harvey, L., Glahn, R.P., 2007. The use of solubility, dialyzability, and Caco-2 cell methods to predict iron bioavailability. Int. J. Vitam. Nutr. Res. 77:158-165.

Garcia-Casal, M.N., Leets, I., Layrisse, M., 2000. Beta-carotene and inhibitors of iron absorption and modify iron uptake by Caco-2 cells. J. Nutr. 130:5-9.

Genc, Y., Humphries, J.M., Lyons, G.H., Graham, R.D., 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. J Trace Elem Med Biol 18: 319-324.

Gibson, R.S., 2006. Zinc: The missing link in combatting micronutrient malnutrition in developing countries. Proc Nutri Soc 65: 51-60.

Glahn R.P., Lee, O.A., Yeung, A., Goldman, M.I., Miller, D.D., 1998. Caco-2 cell ferritin formation predicts non-radiolabelled food iron availability in an *in vitro* digestion/ Caco-2 cell culture model. J. Nutr. 128:1555-1561.

Glahn, R.P., van Campen, D.R., 1997. Iron uptake is enhanced Caco-2 cell monolayers by cysteine and reduced cysteinyl glycine. J. Nutr. 127:642-647.

Graham, R., Senadhira, D., Beebe, S., Iglesias, C., Monasterio, I. 1999. Breeding for micronutrients density in edible parts of staple food crops: Conventional Breeding. Field Crops Res 60: 57-80.

Gyawali, S., Sunwar, S., Subedi, M., Tripathi, M., Joshi, K.D., and Witcombe, J.R., 2007. Collaborative breeding with farmers can be effective. Field Crops Res. 101:88-95.

Hamblin, M.T., Buckler, E.S., Jannink, J.L., 2011. Population genetics of genomics based crop improvement methods. Trends in Genetics 27: 98-106.

Hirschi, K., 2008. Nutritional improvements in plants: Time to bite on biofortified foods. Trends in Plant Science. 13:459-463.

Hirschi, K.D. 2009. Nutrient biofortification of food crops. Annu Rev Nutr 29: 401-421.

Hotz, C., Loechl, C., Lubowa, A., Tumwine, J.K., Ndeezi, G., Nandutu-Masawi, A., Baingana, R., Carriquiry, A., de Brauw, A., Meenakshi, J.V., Gilligan, D.O. 2012. Introduction of β-carotene-rich orange sweet potato in rural Uganda resulted in increased vitamin A intake among children and women and improved vitamin A status among children. J Nutr 142: 1871-1880.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, W., Lu, Y., Weng, Q., Liu, K., Huang, T., Zhou, T., Jing, Y., Li, W., Lin, Z., Buckler, E.S., Qian, Q., Zhang, Q.F., Li, J., Han, B. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42: 961-967.

Hurrell, R., Egli, I., 2010. Iron bioavailability and dietary reference values. Amer. J. Clin. Nutr. 91S: 1461-1467.

Ingvarsson, P.K., Street, N.R. 2010. Association genetics of complex traits in plants. New Phytologist 189: 909-922.

Islam, F.M.A., Basford, K.E., Jara C., Redden R.J., Beebe, S. 2002. Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genet Res Crop Evol 49: 285-293.

Jovani, M., Barbera, R., Farre, R., Martin de Aguilera, E., 2001. Calcium, iron, and zinc uptake from digests of infant formulas by Caco-2 cells. J. Agric. Food Chem. 49:3480-3485.

Kamfwa, K., Cichy, K.A., Kelly, J.D. 2015. Genome-wide assocaition analysis of symbiotic nitorgen fixation in common bean. Theor Appl Genet 128:1999-2017.

Kami, J,A., Gepts, P, 1994. Phaseolin nucleotide sequence diversity in *Phaseolus*. I. Intraspecific diversity in *Phaseolus vulgaris* L. Genome 37: 751-757.

Kennedy, G., Nantel, G., Shetty, P., 2003. The scourge of "hidden hunger": Global dimensions of micronutrient deficiencies. Food Nutr Agric 32: 8-16.

Kiwuka, C., Bukenya, Z.R., Namaganda, M., Mulumba, J.W., 2012. Assessment of common bean cultivar diversity in selected communities of central Uganda. Afr. Crop Sci. J. 20:149-158.

Korte, A., Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS: A review. Plant Methods 9: 29-38.

Kump, K.L., Bradbury, P.J., Wisser, R.J., Buckler, E.S., Belcher, A.R., Oropeza-Rosas M.A., Zwonitzer, J.C., Kresovich, S., McMullen, M.D., Ware, D., Balint-Kurti, P.J., Holland, J.B. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43: 163-169.

La Frano, M.R., Woodhouse, L.R., Burnett, D.J., Burri, B.J. 2013. Biorfortified cassava increases

β-carotene and vitamin A concentrations in the TAG-rich plasma layer of American women. Br J Nutr 110: 310-320.

Low, J.W., Arimond, M., Osman, N., Cunguara, B., Zano, F., Tschirley, D., 2007. A food-based approach introducing orange fleshed-felshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. J Nutr 135: 1320-1327.

McCarthy, M.I., Abecasis, G.R., Cardon, L.R., Godstein, D.B., Little, J., Ioannidis, J.P., Hirschhorn, J.N., 2008. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9: 356-369.

Miklas, P.N., Singh, S.P., 2007. Common bean in genome mapping and molecular breeding in plants, volume 3: Pulses, sugar, and tuber crops. Ed. Kole C. Springer Verlag Berline Heidelberg.

Nestel, P., Bouis, H.E., Meenakshi, J.V., Pfeiffer, W., 2006. Biofortification of staple food crops. In: Symposium on Food Fortification in developing countries. J Nutr 136: 1064-1067.

Nishimuta, M., Kodama, N., Shimada, M., Yoshitake, Y., Matsuzaki, N., Morikuni, E., 2012. Estimated equilibrated dietary intakes for nine minerals (Na, K, Ca, Mg, P, Fe, Zn, Cu, and Mn) adjusted by mineral balance medians in young Japanese females. J Nutr Sci Vitaminol. 58:118-128

Nunez-Gonzalez A., Maiti, R.K., Verde-Star, J., Cardenas, M.L., Foroughbakch, R., Hernandez-Pinero J.L., Moreno-Limon, S., Garcia-Diaz, G., 2002. Variability in mineral profile in seven varieties of beans (*Phaseolus vulgaris* L.) adapted in northeast of Mexico. Legume Research. 25: 284-287.

Okii, D., Tukamuhabwa, P., Kami, J., Namayanja, A., Paparu, P., Ugen, M., Gepts, P., 2014. The genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) germplasm in Uganda. Afr. J. Biotech. 13:2935-2949.

Oraguzie, N.C., Rikkerink, E.H.A., Gardine, S.E., de Silva, H.N., (Editors) 2007. Association mapping in plants. Spinger, NY.

Ortiz-Monasterio, J.I., Palacios-Rojas, N., Meng, E., 2007. Enhancing the mineral and vitamin A content of wheat and Maize through plant breeding. J Cereal Sci 46:293-307.

Petry, N., Boy, E., Wirth, J.P., Hurrell, R.F., 2015. Review: The potential of common bean (*Phaseolus vulgaris* L.) as a vehicle for iron biofortification. Nutrients 7:1144-1173.

Pfeiffer, W.H., McClafferty, B., 2007. HarvestPlus: Breeding crops for a better nutrition. Crop Sci. 47: S88-S105.

Pinstrup-Andersen, P., 2002. Food and agricultural policy for a globalizing world: Preparing for

the future. Amer J Agric Econ 84: 1201-1214.

Poland, J.A., Bradbury, P.J., Buckler, E.S., Nelson, R.J., 2011. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc Natl Acad Sci USA 108: 6893-6898.

Schmutz, J., McClean, P.E., Mamidi, S., Wu, G.A., Cannon, S.B., Grimwood, J., Jenkins, J., Shu, S., Song, Q., Chavarro, C., Torres-Torres, M., Geffroy, V., Moghaddam, S.M., Gao, D., Abernathy, B., Barry, K., Blair, M., Brick, M.A., Chovatia, M., Gepts, P., Goodstein, D.M., Gonzales, M., Hellsten, U., Hyten, D.L., Jia, G., Kelly, J.D., Kudrna, D., Lee, R., Richard, M.M.S., Miklas, P.N., Osorno, J.M., Rodrigues, J., Thareau, V., Urrea, C.A., Wang, M., Yu, Y., Zhang, M., Wing, R.A., Cregan, P.B., Rokhsar, D.S., Jackson, S.A., 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707-713.

Schroder, S., Mamidi, S., Lee, R., Michael R. McKain, M.R, Phillip E. McClean, P.E., Juan M. Osorno, J.M., 2016. Optimization of genotyping by sequencing (GBS) data in common bean (*Phaseolus vulgaris* L.). Mol. Breed. 36:1-9.

Singh, S.P., Gepts, P., Debouck, D.G., 1991. Races of common bean. Econ Bot 45: 379-396.

Tako, E., Rutzke, M.A., Glahn, R.P., 2010. Using the domestic chicken (*Gallus gallus*) as an in vivo model for iron bioavailability. Poult Sci. 89:514-521.

Tapia V., Arredondo, M., Nunez, M.T., 1996. Regulation of Fe absorption by cultured intestinal epithelia (Caco-2 cell monolayers) with varied Fe status. Amer. J. Physiol. 271:G443-447.

Thudi, M., Li, Y., Jackson, S.A., May, G.D., Varshney, R.K. 2012. Current state-of-art of sequencing technologies for plant genomics research. Brief Funct Genomics 11: 3-11.

Tohme, J., Gonzalez, D.O., Beebe, S., Duque, M., 1996. AFLP analysis for genepools of a wild bean core collection. Crop Sci 36: 1375-1384

Welch, R.M., Graham, R.D., 2000. Breeding crops for enhanced micronutrient content. Plant and Soil 245: 205-214.

Wienk, K., Marx, J., Beynen, A., 1999. The concept of iron bioavailability and its assessment. Eur. J. Nutr. 38:51-75.

Wissuwa, M., 2005. Mapping nutritional traits in crop plants. pp 220-241. In Broadley MR, White PJ (editors) Plant nutritional genomics. Blackwell Publishing, Ames, Iowa.

Witte, J.S., 2010. Genome-wide association studies and beyond. Ann Rev Public Health. 31: 9-20.

Yun, S., Habicht, J., Miller, D., Glahn, R.P., 2004. An in vitro digestion/ Caco-2 cell culture system accurately predicts the effects of ascorbic acid and polyphenolic compounds on iron

bioavailability in humans. J. Nutr. 134:2712-2721.

Zhu, C., Gore, M., Buckler, E.S., Yu, J. 2008. Status and prospects of association mapping in plants. Plant Genome 1: 5-20.

Zimmermann, M.B., Hurrell, R.F. 2007. Nutritional iron deficiency. Lancet 370: 511-520.

CHAPTER 1

GENOME-WIDE ASSOCIATION ANALYSIS OF NUTRITIONAL COMPOSITION RELATED TRAITS AND IRON BIOAVAILABILITY IN COOKED COMMON BEAN (*Phaseolus vulgaris* L.) SEEDS

[Manuscript submitted for publication in Molecular Breeding journal]

Genome-wide association analysis of nutritional composition related traits and iron bioavailability in cooked common bean (*Phaseolus vulgaris* L.) seeds

Abbreviations

ADP, Andean diversity panel; BLASTN, Basic local alignment search tool for nucleotides; FeBIO, Iron bioavailability; GBS, Genotyping-by-sequencing; GLM, General linear model; GWAS, Genome-wide association study; LD, Linkage disequilibrium; MLM, Mixed linear model; PCA, Principal component analysis; QTL, Quantitative trait loci; RILs, Recombinant inbred lines; SNP, Single nucleotide polymorphism.

Abstract

Seed nutrients in legumes are important for human health, particularly in developing countries where there is a heavy reliance on plant-based diets, and amongst vegetarians in developed nations. Here we report on the genetic basis underlying the phenotypic variation for protein, zinc, calcium concentrations, and iron bioavailability present in 206 accessions of common bean (*Phaseolus vulgaris* L.) from the Andean Diversity Panel (ADP). We used 8,111 single nucleotide polymorphisms (SNPs) generated with genotyping-by-sequencing (GBS) to examine the allelic variants' associations with seed protein, zinc, and calcium concentrations, and iron bioavailability in the 206 accessions grown over two years in Michigan. Phenotypic variation was identified for each of the traits, with the highest variation (5.4 fold) found for cooked seed iron bioavailability. In addition, significant SNP-trait associations were found for all traits and explained from 6.3 to 13.2 % of the phenotypic variation. These results expand the current understanding of the genetic architecture underlying these complex nutritional quality traits and iron bioavailability in dry beans. Furthermore, marker-trait associations and candidate genes have utility for future nutritional quality breeding efforts to better biofortify common bean through genomics-assisted breeding.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a widely grown and consumed grain legume and is a dietary staple in many regions in Africa and Latin America (Akibode and Maredia 2011; Beebe 2012). Dry beans are nutrient dense and have value as a plant protein source that is high in the amino acid lysine (Messina 2014; Mitchell et al. 2009). Beans are rich in protein, minerals, and vitamins that are often limited in the human diet including iron (Fe), zinc (Zn), calcium (Ca), potassium (K), and folate (Asif et al. 2013; Mudryj et al. 2014). Natural genetic variation exists for the concentration of many of the nutrients in dry beans (Moraghan and Grafton 2001; Pinheiro et al. 2010) thereby presenting an opportunity to develop cultivars with superior nutritional profiles.

Total protein amount and the intake of the complete complement of amino acids is a limiting factor for human growth and development, especially in persons with low consumption of animal products (Millward and Jackson 2004). Protein inadequacy is a problem in many regions of the world but is highest in Eastern and Southern Africa where 37.8 % of the population is at risk of deficiency (Ghosh et al. 2012). Genetic variability for total seed protein concentration in common bean has been reported to range from 22 to 30 % (Ma and Bliss 1978). There is however limited literature on the inheritance of total seed protein concentration in dry bean seed (Duranti and Gius 1997). Many studies have explored certain classes of proteins like phaseolin, lectins, and arcelin with the purpose of characterizing the origin of beans, but not for the purposes of nutritional value (Delaney and Bliss 1991; Ma and Bliss 1978). In one of the few studies that examined the inheritance of total seed protein concentration, quantitative trait loci (QTL) were discovered on chromosomes Pv05 and Pv07, and together these QTL explained 15 to 23 % of the phenotypic variability in protein concentration (Casanas et al. 2013). Since protein is a major limitation in people who rely on beans as a dietary staple, especially in Eastern and Southern Africa,

understanding genetic diversity for total protein concentration warrants consideration during breeding.

Zinc mineral deficiency is a leading public health concern (Gibson 2006) as it affects close to two billion of the world's population (Stein 2010). Zinc malnutrition mainly affects children, pregnant, and nursing women (Black et al. 2008). Insufficient levels of Zn in the human body impairs growth, reduces mental/ cognitive abilities, limits the body's ability to resist infection, as well as the ability to effectively utilize other nutrients from the food matrix (Rodgers et al. 2004). Seed Zn concentration in common bean is quantitatively inherited. Numerous QTL studies have been conducted in inter- and intra- genepool crosses and some genomic regions appear to be important for seed Zn accumulation across mapping populations, most notably on Pv01, Pv02, Pv06, Pv08, Pv10, and Pv11 (Blair et al. 2009a; Blair et al. 2010a; Cichy et al. 2009a). Dry beans are also a rich source of Zn, and two-fold phenotypic variability for seed Zn concentration has been observed (Islam et al. 2002a). Biofortification of beans with increased Zn concentration may therefore be a viable option to improve the Zn status of bean consumers around the world.

Iron (Fe) deficiency afflicts about two billion people around the world and most of the affected populations are resource-limited people living in developing countries (Nestel et al. 2006; Hirschi 2009). The most vulnerable segments of the population for Fe deficiencies in developing countries are women, pregnant and nursing women, and infants and pre-school children (Zimmermann and Hurrell 2007). Fe deficiency in humans can result in mental impairment, high morbidity rates, and physical incapacitation (Boccio and Iyengar 2003; Caballero 2002). Widespread micronutrient malnutrition results in massive negative socioeconomic impact at the

individual and community levels often causing stagnation of national development (Darnton-Hill et al. 2005; Stein 2010).

Common bean biofortification has been deployed as a viable approach to solving iron malnutrition in areas where beans are a staple food source. However, one of the challenges to iron biofortification is not all beans with high iron concentration are efficacious in addressing iron malnutrition in target groups (Petry et al. 2012). Often, iron bioavailability which is a measure of how much iron is available for human absorption following ingestion and digestion is not correlated with seed iron concentration (Elad et al. 2011). While there has been progress on deciphering functionally relevant molecular tags like markers and QTL (Blair et al. 2011) and genes (O'Rourke et al. 2014) underlying Fe concentration, there have been no prior efforts to delineate genomic regions controlling iron bioavailability in common bean.

Potassium is an important nutrient for human health and nutrition. It is involved in reduction of blood pressure, improved urinary sodium excretion, and vascular smooth muscle relaxation (Ekmekcioglu et al. 2016; Houston 2011). Therefore, K is important in the prevention of high blood pressure and hypertension, which are major public health concerns among populations in the Western world. Moreover, K has been reported as one of the major shortfall nutrients in the American diet (USDA and HHS Dietary Guidelines Advisory Committee Report, 2011; Weaver 2013) as well as among people from the United Kingdom (Miller et al. 2016). Dry beans contain high amounts of K and can thus be a useful dietary component of the Western diet (Mitchell et al. 2009). There is however, limited information on the genetic control of K accumulation in common bean. Only one study has explored the genomic regions underlying K accumulation and reported QTL on Pv02 and Pv07 with each QTL explaining 23.2 % and 24.2 %

of the accumulation in K respectively in the DOR364 x G19833 common bean mapping population (Blair et al. 2016).

Calcium in foods is important for bone building and bone function in the human body (Bass and Chan 2006; Rizzoli et al. 2008) and it is particularly important for Zn absorption. Quantitative trait loci on Pv01, Pv07, and Pv09 have been reported to be associated with Ca concentration in a common bean mapping population (Casanas et al. 2013). Guzman-Maldonado et al. (2003) reported QTL for seed Ca concentration on Pv01 and Pv04 in an $F_{2:3}$ common bean mapping population. As with other minerals, previous research has also established a genetic basis for the phenotypic variation observed in Ca concentration in common bean seeds, which again presents an opportunity to increase the concentration through plant breeding.

Phytic acid (*myo-inositol* 1,2,3,4,5,6 hexakisphosphate), the major form of phosphorus (P) storage in bean seeds, has the capacity to bind iron and zinc from the food matrix, thus making them unavailable for absorption in the gut (Brinch-Pedersen et al. 2007; Lott et al. 2000). Quantitative trait loci for P concentration in bean seeds have been identified on several chromosomes. Cichy et al. (2009b) working with an $F_{5:7}$ RIL population reported QTL for seed P concentration on Pv01, Pv02, Pv05, Pv06, Pv08, and Pv11 and QTL for phytic acid were found on Pv04 and Pv08. In both cases, the QTL explained about 17-55 % of the phenotypic variation. In addition, QTL have been reported on Pv02, Pv06, and Pv11 for total seed phytate and seed P in an inter-genepool common bean population with R^2 values of 18-29 % (Blair et al. 2009b). Mutation breeding has also been explored to develop low phytic acid phenotypes in common bean (Campion et al. 2009) and the resulting mutants have exhibited improved iron bioavailability with stable and acceptable agronomic performance (Campion et al. 2013). Using QTL mapping experiments in segregating RIL populations (Blair et al. 2012) and *in silico* mapping (Fileppi et al.

2010), candidate genes involved in the phytic acid biosynthesis pathway have been mapped in the common bean genome and functional markers have been developed (Panzeri et al. 2011).

Recent advances in dry bean genomics, particularly the development of the Illumina BARCBean6K_3 BeadChip (Song et al. 2015), the ability to generate GBS SNP datasets (Elshire et al. 2011; Schroder et al. 2016), and the sequencing of the first chromosome-scale common bean reference genome (Schmutz et al. 2014) have enabled genome-wide association study (GWAS) to be an attractive approach for examining the genetic architecture of polygenic traits in common bean. The approach is based on the phenotyping and genotyping of diverse germplasm collections and relies on historical recombination events that have accumulated during crop evolution, domestication, and crop improvement. Genome-wide association studies have the potential to resolve marker-trait associations and in some cases, to identify causal genotype-phenotype relationships that condition qualitative and quantitative trait variation (Zhu et al. 2008; Ingvarsson and Street 2011). Given the potential of GWAS to help identify, understand, and develop molecular markers to track important allelic variation for mineral nutrition status and the importance of common bean to the nutrition of millions of people around the world, there is clearly a need to explore and dissect the genetic architecture of nutritional quality traits in common bean. Therefore, the main objective of the research presented here was to identify superior germplasm as well as the genomic regions that are associated with cooked seed nutrient concentrations via GWAS in a previously developed and characterized ADP (Cichy et al. 2015a; Cichy et al. 2015b; Kamfwa et al. 2015). We utilized data from cooked seed samples, as these better represent actual concentrations that would be available to consumers after processing as compared to raw seed.

Materials and Methods

Field site characteristics

The plant materials were grown at Michigan State University's Montcalm Research Center near Entrican MI, USA and the field experimental design was as described previously (Cichy et al. 2015b). The plant materials were grown for two years during the summers of 2012 and 2013. Soil chemical tests were conducted at the time of planting by the Michigan State University Soil and Plant Nutrient Laboratory. In 2012, the soil nutrient levels in the experimental plots were 232 µg g^{-1} of P, 189 µg g^{-1} of K, 965 µg g^{-1} of Ca, 2.5 µg g^{-1} of Zn, and 34.4 µg g^{-1} of iron. The soil organic matter was 1.2 % and the soil pH was 6.1. In the 2013 growing season, the trial site contained 298 µg g^{-1} of P, 169 µg g^{-1} of K, 594 µg g^{-1} of Ca, 5.2 µg g^{-1} of Zn, and 95.1 µg g^{-1} of iron. The soil organic matter was 2.5 % and the soil pH was 6.5.

Germplasm and field plot design

A total of 206 Andean common bean genotypes, a subset of the ADP (characterized in Cichy et al. 2015a) were used for this study. Geographically, the study subset was composed of 100 accessions from Africa, four from Europe, 12 from the Caribbean, five from Central America, four from Europe, 71 from North America, and 10 from South America. Of these 206 ADP genotypes, 108 were landraces, 72 were varieties, and 26 were breeding lines. The seeds were planted in two-row plots, with 80 seed per row, in a randomized complete block design with two replications. Within row spacing was about four seeds per 30.5 cm and between row spacing was 0.5 meters. At maturity, the two-row plots were hand harvested and the plants were threshed using a Hedge 140 plot harvester (Wintersteiger, UT, USA).

Phenotyping of nutritional quality traits

Preparation and cooking of seed for nutritional analysis

Harvested seed lots were cleaned of split and broken seed and were then maintained in a humidification chamber lined with one liter of saturated sodium chloride solution for one month. The saturation protocol was performed to ensure that the seeds were at comparable moisture levels prior to cooking and subsequent nutrient analysis. After one month, the seed moisture was at 10-14 % and 25 seeds were cooked using an established Mattson cooker protocol (Wang and Daun 2005). The Mattson cooker assay has also been recently applied to characterize the variability in cooking time present in Andean beans as well as the underlying genetic architecture for this trait by Cichy et al. (2015b). Following cooking, seed were placed in 50mL tubes and held at -20°C until freeze-dried (Cichy et al. 2015b). Freeze-dried samples were then pulverized with marble beads and a Geno/Grinder to a fine consistency (SPEX SamplePrep, Inc). The homogenized powder for each sample was divided into appropriate amounts for each nutritional trait analysis.

Cooked seed protein measurement

In both 2012 and 2013, the cooked seed samples were shipped to A & L Great Lakes Laboratories, Inc. for seed nitrogen analysis. Total seed nitrogen was determined using the Kjeldahl method (Bremner 1965). Total seed nitrogen was multiplied by a conversion factor of 6.25 in order to determine the percent protein in the cooked bean samples (AOAC 1980).

Quantification of cooked seed zinc, iron, potassium, calcium, and phosphorus concentrations

In 2012 the cooked bean samples were shipped to A and L Great Lakes contract lab in Fort Wayne, IN, USA. Seed from the 2013 experiment were shipped to the USDA-ARS Children's

Nutrition Research Center in Houston, TX (former institution of M.A. Grusak) for the nutrient analyses. Across both labs, inductively coupled plasma – optical emission spectroscopy was used to quantify minerals and their extraction from the cooked bean samples was as described previously (Farnham et al. 2011). Nutrient concentration values in this study are presented on a dry weight basis.

Quantification of iron bioavailability

In both 2012 and 2013, iron bioavailability (FeBIO) was quantified using an established *in vitro* Caco-2 cell line assay (Glahn et al. 1998). Briefly the protocol involves sample preparation (cooking, lyophilization, pulverization, and weighing out of 0.5 mg for each replicate), a two-step enzyme digestion (pepsin and pancreatin-bile) simulating gastric and intestinal digestions, then the resulting digests are passed through a dialysis membrane connected to the Caco-2 cell monolayers bathed in a culture medium. Following a 24-hour period post-pancreatin-bile digestion, the Caco-2 cells were harvested and processed to determine ferritin concentration. Caco-2 cells adjust to elevated levels of intracellular Fe concentration by synthesizing more ferritin. This makes ferritin formation a powerful biomarker for quantifying Fe absorption by the Caco-2 cell monolayers. The ratio of ferritin/total Caco-2 cell protein (expressed as ng ferritin/ mg protein) was used as an index for cellular Fe uptake. The assay for each sample was conducted in triplicates and ranked against a Merlin navy bean control. Final iron bioavailability values for each of the 206 ADP accessions were reported as a percentage of the Merlin navy bean. The Merlin navy bean was used as a control since white beans have been reported to exhibit the highest levels of iron bioavailability (Tako and Glahn 2011).

Determination of seed phytic acid

Phytic acid was determined using Wade's reagent colorimetric method (Gao et al. 2007) with some minor modifications. A 200 µL aliquot for all the genotypes and standards were pipetted into 96-well microtiter plates and absorbance values at 500 nm were captured using a microplate reader (BioTek, Inc). A phytic acid sodium salt hydrate standard (Sigma Aldrich Co) was used to prepare standard curves for each plate reading, and the values of phytic acid phosphorus (PA-P) in the bean samples were determined and expressed in milligrams per gram of bean powder following previously established conversions (Latta and Eskin, 1980). To convert PA-P to phytic acid concentration, we assumed that phytic acid (660 g $mole^{-1}$) contains six moles of phosphorus (31 g $mole^{-1}$), implying that a total of 186 g of phytic acid phosphorus are in one mole of phytic acid (Raboy et al.1984). All our PA-P values in mg g^{-1} of bean powder were then multiplied by a conversion factor of 660/186 (or 3.548387) to estimate mg of phytic acid per g of cooked bean powder.

Genotyping-by-sequencing of the Andean diversity panel

DNA isolation and quantification

Genomic DNA for GBS library construction was isolated by harvesting ~50 mg of leaf tissue (~1cm^2) from actively expanding trifoliolate leaves, lyophilizing for 48 h, grinding to a fine powder using silica beads (OPS Diagnostics) and a TissueLyser II homogenizer (Qiagen), and then by using a DNeasy 96 Plant Kit (Qiagen) according to the manufacturer's instructions. The DNA was eluted into a total volume of 40 µL. Double-stranded DNA was quantified with the QuantiFluor dsDNA Dye System (Promega) and a Quantus Fluorometer (Promega) according to the manufacturer's instructions. Genomic DNA from each sample was diluted to 5 ng μL^{-1} with nuclease free water and arrayed in PCR plates in preparation for GBS library construction.

GBS library construction and sequencing

Two GBS libraries were constructed to genotype 500 accessions of an expanded ADP, one library at 364-plex, and the second library at 137-plex (one accession duplicated). The DNA samples for each of the accessions were assigned a barcode-adapter according to the key file presented in Supplemental Table S1. The GBS libraries were based on *Ape*KI complexity reduction and were constructed using previously published protocols, barcode-adapters, and PCR primers (Elshire et al. 2011) as optimized for use with *P. vulgaris* where 1.5 ng of each adapter is used per 50ng of sample DNA (Hart and Griffiths 2015). The GBS libraries were quantified and validated on an Agilent 2100 Bioanalyzer (Agilent Technologies), diluted, and sequenced by 101-cycle single-end sequencing on an Illumina HighSeq 2500 instrument (Illumina) at the Weill Cornell Medical College Genomics Resources Core Facility. The 364-plex library was sequenced on five lanes and the 137-plex library was sequenced on two lanes. Raw sequencing data was deposited in the NCBI Sequence Read Archive as study accession SRP061551.

Sequence processing, alignment, SNP genotyping

Raw sequencing reads were processed with the GBS Discovery Pipeline (Glaubitz et al. 2014) as implemented in TASSEL version 3.0.168 (Bradbury et al. 2007). High-quality unique sequence reads that contained a barcode, the *Ape*KI cut site, and a genomic sequence insert (termed GBS tags) were aligned to the *Phaseolus vulgaris* v2.0 reference genome assembly (Schmutz et al., 2014) by implementing the Burrows-Wheeler Aligner (Li and Durbin, 2009). The Discovery SNP Caller of TASSEL v3.0.168 was implemented to call SNPs and was directed to impose a minimum inbreeding coefficient of 0.9 and a minimum minor allele frequency (MAF) of 0.05. This SNP data was further filtered to eliminate SNPs with more than 50% missing data and to eliminate SNPs that were not in statistically significant linkage disequilibrium (R^2=0.01) with at

least one neighboring SNP within a 50-SNP window. Missing data was imputed by implementing Fast Inbred Line Library ImputatioN (Swarts et al. 2014) in TASSEL v5.0.9 using default settings. This resulted in a dataset of 13,202 SNPs called across the 500 ADP accessions.

Linkage disequilibrium, population structure, and kinship analyses

The original GBS dataset of 13,202 SNP markers was filtered in TASSEL v5.2 (Bradbury et al. 2007) for missing data and MAF of 0.05 to generate a new dataset containing 8,111 SNP markers for the 206 accessions that were phenotyped for the seed nutritional quality traits in this research. This dataset was then used for all of the downstream analyses including the assessments of linkage disequilibrium, population structure, and kinship as well as for GWAS. Linkage disequilibrium among markers was computed using the full matrix option in TASSEL v5.2 (Bradbury et al. 2007) and all heterozygotes were set as missing. This is reasonable because common bean is a highly inbred crop (Ibarra-Perez et al. 1997). Linkage disequilibrium (LD) was estimated as the squared allele frequency correlation (r^2) between pairs of markers for every chromosome. To examine and control for population stratification across the ADP, we used principal component analysis (Price et al. 2006) implemented in TASSEL v5.2 (Bradbury et al., 2007). The generated principal components were used as covariates in the genome-wide marker-trait association analysis to account for population structure. To account for relatedness within the panel we computed a kinship coefficient matrix for all genotypes using the scaled identity-by-state method (Endelman and Jannink 2012) also implemented in the software TASSEL v5.2 (Bradbury et al. 2007).

Phenotypic data analysis

Pearson correlation coefficients among traits were determined using PROC CORR command in statistical analysis software, SAS 9.4, (SAS-Institute-Inc 2011). To minimize environmental effects on the distribution of the phenotypes, best linear unbiased predictors (BLUPs) were generated for all traits across years and replications using the lme4 package (Bates et al. 2015) in the R environment. The trait BLUPs for each accession were combined and used as the phenotypic input file for TASSEL v5.2 to perform GWAS. The statistical model that we used to generate BLUPs and conduct analysis of variance ANOVA is: $Y_{ijk} = \mu + G_i + Y_j + GY_{ij} + B(Y)_{jk} + \varepsilon_{ijk}$

where Y_{ijk} is the measured phenotypic value of the i^{th} genotype in the k^{th} replication of the j^{th} year, μ is the grand mean, G_i is the effect of the i^{th} genotype, Y_j is the effect of the j^{th} year, GY_{ij} is the interaction between the i^{th} genotype and the j^{th} year, $B(Y)_{jk}$ is the effect of the k^{th} replication within the j^{th} year, and ε_{ijk} is the error term assumed to be normally distributed with mean = 0.

Genome-wide marker-trait association analysis

To minimize the occurrence of false positives (SNP marker *P* value inflation), we tested the power of two statistical models: a general linear model (GLM), which is a simple model, and a more conservative mixed linear model (MLM). The GLM model uses P+Q where P is the matrix of phenotypic values or BLUPs in our study and Q is the matrix of population structure covariates generated from principle component analysis (PCA). The MLM model incorporates both the fixed and random variables/effects in the GWAS, i.e. P+Q+K where P are the BLUP values, Q is population structure covariates, and K is the kinship matrix for cryptic relatedness among the 206 accessions. For the MLM we used the efficient mixed model association (EMMA) and population parameters previously determined (P3D) algorithms implemented in TASSEL v5.2 to estimate

marker-trait associations and the variance components as these have been developed to be computationally efficient (Zhang et al. 2010). The MLM model included the GBS SNP markers and the population structure matrix from the principal component analysis (PCA) as fixed effects while the relative kinship matrix was included as a random effect (Yu et al. 2006). The mathematical notation of the simple model or GLM equation was: $Y = X\alpha + P\beta + \varepsilon$ while for MLM, the equation was: $Y = X\alpha + P\beta + K\mu + \varepsilon$ where in both methods Y is the vector for best linear unbiased predictors for every trait, (for example, seed protein concentration of 25 cooked seeds), X is the vector of SNP effects, P is the vector of the population structure effects from the PCA, K is the vector for the kinship effects, and ε is the vector for the residuals assumed to be normally distributed with mean = 0. To estimate the proportion of phenotypic variability explained by each significant marker we used the R^2 values generated in MLM statistics output from TASSEL v5.2 software. The SNP marker effects on all traits were reported based on the allelic effects table outputted from TASSEL. We examined the Quantile-Quantile (QQ) plots from both GLM and MLM analyses to determine how well both models fit our GWAS data while minimizing inflation of the genetic marker *P* values (false positives). The QQ plots show graphical visualizations of the distribution of expected (X) vs. the observed (Y) *P* values along the diagonal X=Y line with a sharp curve towards the end that is representative of true and significant marker-trait associations. For both GLM and MLM, correction for multiple testing was carried-out using *q*FDR in the QVALUE v3.5 R package. The *q*FDR is an extension of the false discovery rate (FDR) method (Benjamin and Hochberg, 1995) that employs a smoother option when determining *P* value cutoffs (Storey and and Tibshirani 2003). The GBS SNP markers with *q*FDR values <0.01 were declared significant. To visualize the SNP-trait associations from the GLM and MLM analyses, the chromosome and physical positions, and *P* values for each SNP for all traits were

combined and formatted for use as an input file for a script written in R (Turner 2014) to generate the QQ and Manhattan plots.

Candidate gene identification

To detect putative candidate genes associated with the significant SNPs, we used the JBrowse feature of Phytozome (Goodstein et al. 2012) to examine the *P. vulgaris* v2.1 genome (Schmutz et al. 2014) for genes relevant to nutritional quality traits in common bean. While in Phytozome, we examined the immediate 1.0 Mb genomic region upstream and downstream (± 1.0 Mb) of every significant SNP to pinpoint plausible candidate genes. Due to the high self-pollinated nature of common bean (Ibarra-Perez et al. 1997), linkage disequilibrium (LD) decays relatively slowly and LD estimates can reach up-to 6 Mb for some chromosomes. The mean genome-wide LD decay blocks in our panel of 206 ADP accessions extended across a 2.5 Mb genomic region and thus the reason why we used the 1.0 Mb search window on either side of the significant marker signals to scan for plausible candidate genes. For genes with unknown functional annotations, we conducted a BLASTN search (Altschul et al., 1997) using the *P. vulgaris* gene sequence as a query against the *Arabidopsis thaliana* L. Heynh. genome in TAIR (Rhee et al. 2003), the *Glycine max* L. Merr. genome (Schmutz et al. 2010), and /or *Oryza sativa* L. genome (Matsumoto et al. 2005) at the National Center for Biotechnology Information website. We declared a gene as a plausible candidate if it met the following criteria: (a) the gene had a known function that related to the trait being evaluated based on gene ontology term descriptions in Phytozome; (b) BLASTN searches from *Arabidopsis*, soybean, and /or rice genomes returned homologous sequences that had functions relevant to our phenotypes of interest; (c) a gene contained or was in close proximity to the significant SNP in the search region.

Results

Phenotypic trait summary statistics

Nutritional quality trait characterization was conducted on cooked bean samples. Over both field seasons (2012 and 2013) analysis of variance indicated significant effects of genotypes on the measured traits at a *P* value <0.0001. Seed protein concentration ranged from 20.4 to 27.5 % with a 1.4 fold variation (Table 1.1). The accession UCD0906 from the USA had the highest seed protein concentration of 27.5 %. Both seed Zn and Fe had a 1.8 fold variation in the ADP germplasm where Kamiakin a light red kidney from USA had the highest levels of Zn of 39.7 µg g^{-1}. The red mottled genotype PR-12 from Puerto Rico had the highest amount of Fe concentration of 99.3 µg g^{-1}. Iron bioavailability varied from 20.4 to 110.7 % of Merlin navy bean (or a 5.4 fold variation). Yellow beans had a pattern of uniquely higher amounts of FeBIO where genotypes like Myasi from USA, Chumbo Cela and Ervilha from Angola, and PI319706 from Tanzania had FeBIO values of 82 % or greater.

Potassium ranged from 5.56 to 11.1 mg g^{-1} while Ca varied from 0.7 to 2.1 mg g^{-1}. The light red kidney genotype ND061106 (BC-315) from USA had the highest concentration of seed K (11.1 mg g^{-1}) but also ranked second in seed protein concentration with a value of 27.4 %. Seed phosphorus and phytic acid concentrations had 1.8 and 1.6 fold variation respectively. Small red colored ADP genotypes were predominantly low in seed phosphorus and phytic acid concentrations and these might be useful assets for breeders striving to develop common bean with the low phytic acid phenotype. For instance, a small red Kiboroloni (TZ-11) from Tanzania had the lowest levels of seed P (3.2 mg g^{-1}). Another small red genotype Kidungu (TZ-3) from Tanzania had the lowest concentration of seed phytic acid (12.6 mg g^{-1}). Overall, seed protein and phytic acid had the narrowest variation while FeBIO had the widest variability in the ADP.

Seed protein concentrations in the ADP were skewed toward higher levels based on the histogram (Figure 1.1). Seed Zn, Fe, and K concentrations exhibited a normal distribution around the mean, while FeBIO was left skewed (Figures 1.1 and 1.2). Seed Ca, phosphorus, and phytic acid were right skewed from the mean (Figure 1.2). The histograms showed the quantitative inheritance nature of these nutritional quality traits with potentially multiple loci involved in uptake, accumulation, homeostasis, and/or retention (following cooking).

The analysis of variance (ANOVA) revealed that over 50 % of the variability in seed protein and Zn concentration was due to the genotype effects followed by the interactions among genotypes and years (Table 1.2). Variability in seed Fe and FeBIO was mostly controlled by the genotype at 50.2 and 40.2 % of TSS respectively. Interactions between genotype and year were the second largest contributors of variability in seed Fe and FeBIO at 16.2 and 22.6 % of TSS respectively (Table 1.2). Seed K was largely controlled by effects of the year (40.2 % of TSS) followed by genotype at 36.1 % TSS of squares. Over 50 % of TSS explained the variability in seed Ca and P (Table 1.3). Seed phytic acid was strongly controlled by the environment at 54.4 % of TSS followed by effects of genotype at 23.6 % of TSS (Table 1.3).

Phenotypic correlations

Over the two years, most traits were positively correlated with each other except for seed Ca concentration and significant correlations ranged from 0.24 to 0.76 (Table 1.4). At a *P* value of 0.001, seed protein was positively correlated with Zn (r=0.50), Fe (r=0.50), K (r=0.45), P (r=0.58), and phytic acid (r=0.40). At a *P* value of 0.01 seed protein was negatively correlated with FeBIO (r=-0.20). At a *P* value of 0.001, seed Zn was positively correlated with Fe (r=0.59), K (r=0.32), phosphorus (r=0.60), and phytic acid (r=0.45). At a *P* value of 0.001, Fe was positively correlated K (r=0.29), phosphorus (r=0.45), and phytic acid (r=0.33). At a *P* value of 0.001, FeBIO was

positively correlated with K (r=0.41), phosphorus (r=0.24), and phytic acid (r=0.26). Iron bioavailability was not statistically correlated to Fe, Zn, and Ca at any of the probability test levels for significance (Table 1.4). Potassium was positively correlated to phosphorus (r=0.76) and phytic acid (r=0.54) at a *P* value of 0.001. Calcium was positively correlated to phytic acid (r=0.28) at a *P* value of 0.001. Phosphorus was positively correlated to phytic acid (r=0.74) at a *P* value of 0.001. Overall, 13 of the trait combination tests resulted in moderately strong positive correlations (r= 0.4 to 0.75), eight of the combinations were moderately weak positive correlations (r= 0.19 to 0.39), and seven of the trait combination tests were not significant at any of the three probability levels used to declare significance.

Population structure and genome-wide marker-trait associations

Population stratification in this association mapping panel has been described previously (Cichy et al. 2015a; Kamfwa et al. 2015) where two clusters/ sub-populations were observed among the ADP accessions. Analysis of principal components revealed two clusters as reported in Kamfwa et al. 2015. The small cluster had 10 accessions that were admixtures. Five were landraces, two were breeding lines, and three were varieties. The large cluster contained 196 accessions that belong to the Andean genepool and are from various dry bean production areas of the world. In common bean, population differentiation typically arises from gene pool structure (Andean vs. Middle American), race structure within gene pools, and admixtures resulting from inter-gene pool or inter-racial hybridizations (Kwak and Gepts, 2009). Our study purposely targeted the large seeded Andean gene pool and the small number of 10 admixtures could have arisen from inter-gene pool crosses that breeders often make to improve dry bean germplasm for various traits. To account for this stratification, we used five principal components that were included as covariates in the model for all the association analyses as described in the materials

and methods section. The five principal components cumulatively accounted for 38 % of the genetic variation across the 206 ADP accessions. The SNP markers were tested for their significant associations with all the four traits presented in this study below.

Seed protein concentration

Two of the top five markers for seed protein concentration were located on Pv06 and Pv03 with one on Pv07 (Table 1.5, Figure 1.3C) and explained from 6.3 to 11.6 % of the phenotypic variability in seed protein concentration. The peak SNP (S6_26904824) on Pv06 had a MAF of 0.07 and explained 11.6 % of the variability in protein concentration in the ADP. The second most significant SNP (S7_7597332) on Pv07 had a MAF of 0.25 and an R^2 of 6.3 % as variance explained. The third most significant SNP on Pv03 had a MAF of 0.41 and explained 7.9 % of the phenotypic variability. The remaining two peak SNPs were on chromosomes Pv06 (S6_25757671) and Pv03 (S3_43953289) with a MAF of 0.06 and 0.44 respectively. These genetic markers S6_25757671 and S3_43953289 accounted for 7.7 % and 7.4 % of the phenotypic variability in the ADP germplasm respectively.

Seed zinc concentration

Four of the top five genetic markers for seed Zn concentration were located on the left arm of chromosome Pv07 indicating the importance of this genomic region in seed Zn concentration (Figure 1.4C). The markers explained 7.2 % to 10.4 % of the variability in Zn concentration. The peak SNP on Pv07 (S7_80695) had a MAF of 0.19 and an R^2 value of 9.5 % as the proportion of variability explained. Two other SNPs (S7_338865 and S7_338867), both had a MAF of 0.37 and an R^2 value of 9.5 % as the variance explained (Table 1.5). Another SNP on Pv07 (S7_295275) had a MAF of 0.23 and explained 10.4 % of the variability in Zn concentration. The SNP marker on Pv10 (S10_2707473) had a MAF of 0.23 and an R^2 of 7.2 % as the proportion of the variability

explained. The peak genetic marker S7_80695 was in LD with markers S7_338865 and S7_338867 (r^2=0.38; *P* value=5.14 x 10-^{20}). This marker was also in LD with SNPs S7_338868 (r^2=0.39; *P* value=8.16x10^{-20}) and S7_295275 (r^2=0.69; *P* value=6x10^{-25}). The SNP marker S7_295275 was in LD with SNPs S7_338865 and S7_338867 (r^2=0.41; *P* value=3.61x10^{-17}) as well as SNP S7_338868 (r^2=0.42; *P* value=4.84x10^{-17}). Most of the five peak SNPs on Pv07 were in significant LD with each other implying they might be tagging the same candidate gene or suite of genes involved in the regulation of seed Zn concentration in common bean.

Seed calcium concentration

The SNP markers on Pv01, Pv02, Pv04, and Pv11 were associated with Ca concentration and had R^2 values between 8 % and 10 % as proportion of variance explained (Table 1.5, Figure 1.5C). The peak SNP on Pv11 (S11_7613114) had a MAF of 0.31 and explained 9.8 % of the variability in Ca concentration. The next two peak SNPs were on Pv02 (S2_14889467 and S2_28089586) and had a MAF of 0.08 and 0.06 respectively. These markers also explained 8.6 % and 8.3 % of the variability in Ca concentration respectively. The SNP marker on Pv04 (S4_47502132) had a MAF of 0.25 and explained 8.2 % of the variability in calcium concentration. The peak SNP located on Pv01 (S1_48843183 had a MAF of 0.06 and explained 8.1 % of the variability in Ca concentration. The peak SNPs on Pv02 (S2_14889467 and S2_28089586) were in LD with each other (r^2=0.38; *P* value=2.27x10^{-09}).

Iron bioavailability

There were significant marker-trait associations for FeBIO on Pv06, Pv07, and Pv11 that explained about 8.3 % to 13.2 % of the variability in the ADP (Table 1.5, Figure 1.6C). The peak SNP on Pv11 had a MAF of 0.07 and explained 13.2 % of the variability in FeBIO. There were

two significant SNPs on Pv06 (S6_28278333 and S6_23708741) with a MAF of 0.48 and 0.05 respectively. They explained 9.8 % and 8.4 % of the variability in FeBIO in the ADP germplasm. Another SNP marker on Pv07 (S7_36385041) had a MAF of 0.33 and explained 8.3 % of the variability in FeBIO. The fifth SNP was on chromosome Pv06 with a MAF of 0.36 explained 8.8 % of the variability in FeBIO in the ADP. The peak SNPs on Pv06 (S6_28278333 and S6_27783532) were in LD with each other (r^2=0.30; *P* value=$1.67x10^{-15}$). There were no marker-trait associations uncovered by GWAS in our study for seed K, Fe, P, and phytic acid.

Discussion

In this study, we conducted GWAS for protein, Zn, and Ca concentration and FeBIO in cooked seeds of common bean using both GLM and MLM statistical models. The two methods indicated that including both population structure and kinship covariates significantly reduces marker *P* value inflation or false positives (Figures 1.3 to 1.6 Panels B and D). We thus used marker-trait associations from MLM analysis for significant markers and candidate gene searches (Table 1.5; Figures 1.3 and 1.6 Panel C). Previous work on the genetic architecture of Zn and protein accumulation in common bean have used bi-parental mapping (Blair et al. 2009a; Blair et al. 2010b; Blair et al. 2011; Blair et al. 2016; Cichy et al. 2009a) or advanced back-cross QTL mapping procedures (Blair and Izquierdo 2012) on dry raw seeds. Unlike previous reports, this study is unique and more representative of real conditions because all the trait measurements were conducted on cooked bean seeds, and thus better represent the nutritional composition of dry beans consumed as staple foods.

There was significant genetic variation for all traits measured in our study. This range of values will be useful for selection and improvement of Andean beans for nutritional quality traits through both traditional and molecular breeding. The ADP germplasm had high levels of protein

with concentrations upto 27.5 %. There is limited information on the genetic architecture for protein concentration in common bean as only Casanas et al. (2013) has reported QTL for protein concentration in common bean. Seed Zn concentration ranged from 21.6 to 39.7 µg g^{-1} and this distribution is in agreement with results reported previously for uncooked beans (Cichy et al. 2009a; Islam et al. 2002a; Islam et al. 2002b). Seed Fe concentration ranged from 54.5 to 99.3 µg g^{-1} that is typical for common bean (Islam et al. 2002a). Iron bioavailability varied from 20.3 to 110.7 % of Merlin navy bean. This is the first effort to evaluate this trait in a large and diverse set of common bean germplasm of any genepool. Our results will serve as a benchmark for future efforts on further understanding the inheritance as well as effects of molecular tags and environment on the penetrance of FeBIO. Seed K and Ca concentrations ranged from 5.6 to 11.1 mg g^{-1} and 0.7 to 2.1 mg g^{-1} of bean sample respectively, which are typical values for these minerals in seeds, especially for plants that tend to accumulate large amounts of K and Ca (White 2013; White and Broadley 2003). The results from this study showed high levels of phosphorus and phytic acid concentrations as has been reported previously for uncooked bean seeds (Blair et al. 2009b; Blair et al. 2012; Cichy et al. 2009b). Legumes, including dry beans, typically tend to have high levels of phytate or phytic acid (Lott et al. 2000; Raboy 2007). Phosphorus, which is typically stored as phytic acid in legumes and cereals (Lott et al. 2000; Raboy 2007) was positively correlated to phytic acid.

The bell shaped curve of the histograms (Figures 1.1 and 1.2) demonstrate the quantitative inheritance nature of these nutritional quality traits and suggest that multiple loci might be involved in the homeostasis, accumulation, and/or retention of the studied traits. Most nutrients, particularly protein, Zn, Fe, FeBIO, K, P, and phytic acid concentration, were positively correlated. This

implies that simultaneous selection for these phenotypes is feasible in common bean nutrition breeding and improvement programs.

Marker-trait associations

There were significant marker-trait associations for seed protein on Pv03, Pv06, and Pv07. Casanas et al. (2013) reported QTL for seed protein on Pv05 and Pv07 using SSR and AFLPs markers. Kamfwa et al. (2015) reported QTL for nitrogen associated traits (nitrogen derived from the atmosphere and percent nitrogen derived from the atmosphere in the shoot) on Pv07 and Pv11.

Our results also indicated the presence of QTL for Zn concentration on Pv07 and Pv10. The significant markers on these chromosomes explained about 7.2 to 10.4 % of the phenotypic variability in Zn levels. One candidate gene tagged by a marker on Pv10 and relevant to Zn ion binding and homeostasis in plants were also discovered. Previous QTL mapping have found regions associated with Zn on Pv01, Pv02, Pv05, Pv06, Pv08, Pv10, and Pv11 with these QTL explaining up-to 39 % of the variation in seed Zn concentration (Cichy et al. 2009a). Blair et al. (2009a), using an inter-genepool RIL population (DOR364 x G19833), found QTL for seed Zn concentration on Pv03, Pv06, Pv07, Pv09, and Pv11 with R^2 values ranging from 6 to 29 %. In another bi-parental mapping population using parents belonging to the Mesoamerican genepool (G14519 x G4825), QTL for seed Zn were found on Pv01, Pv02, Pv03, Pv06, and Pv08. These QTL had R^2 values ranging from 10 to 38 % (Blair et al. 2010a). In another QTL mapping experiment working with a population within the Andean bean genepool (G21242 x G21078), three QTL on Pv02, Pv07, and Pv08 were discovered and these genomic regions explained the variation in Zn accumulation with R^2 values ranging from 13 to 27 % (Blair et al. 2011). These past efforts along with our study indicate that seed Zn concentration is controlled by multiple genes distributed across several chromosomes of the common bean genome.

There were significant markers associated with seed Ca concentration on Pv01, Pv02, Pv04, and Pv11. The two most significant markers (S11_7613114 and S2_14889467) explained 9.8 % and 8.6 % of the variability in Ca levels in the cooked seed respectively. The SNP marker S1_48843183 tagged three genes encoding for calmodulin or calcium transporter proteins (Table 1.5). Blair et al. (2016) working on a bi-parental mapping population derived from a cross of DOR364 x G19833 found a QTL for Ca accumulation on Pv08, while Casanas et al. (2013) found QTL for Ca concentration on Pv01, Pv07, and Pv09 using a RIL population from a cross between Xana x Cornell 49242. The QTL information on Ca concentration in common bean is still scarce and the two studies did not agree on chromosomes carrying the genomic regions underlying the seed Ca trait. Our new QTL on Pv01, Pv02, Pv04, and Pv11 need to be further examined and validated in other mapping populations before these markers can be deployed in marker assisted selection efforts for calcium improvement in common bean. Iron bioavailability QTL were identified on Pv06, Pv07, and Pv11 that explained between 8 – 14 % of the variability observed for this trait in the ADP.

Favorable marker allelic classes in the ADP germplasm

Favorable allelic combinations between marker loci for seed protein and Zn concentrations and FeBIO across peak loci were assessed to determine their distribution across the ADP accessions and their potential use in pyramiding to improve common bean for the above traits. We used the peak SNPs for protein, Zn, and FeBIO data where we had significant marker-trait associations. The peak SNP for protein concentration was S6_26904824 with alleles C and T. The minor allele frequency for this variant was 0.07 and the minor allele was T (Table 1.5). The C major allele resulted in a slightly lower amount of protein concentration. On average homozygous CC accessions had 22.3 % protein while the homozygous TT individuals accumulated 25.5 % protein in the seed per plant. For Zn concentration, the peak SNP was S7_80695 and had an R^2

value of 9.55 %. The peak SNP had alleles G and A with G as the major allele and A as the minor allele (MAF=0.19, Table 1.5). The major allele resulted in a higher seed Zn concentration, where on average homozygous GG accessions had 36.7 µg g^{-1} while the AA genotypic class had 30.5 µg g^{-1} of Zn in seeds. For seed FeBIO, the peak SNP was S11_1331783; this SNP had alleles C and G with a minor allele frequency of 0.07 (Table 1.5). The major allele was C, while the minor allele was G. The genotypic class CC resulted in a mean of 86.8 % of Merlin navy bean as FeBIO while the genotypic class of GG resulted in 57.5 % of Merlin navy bean as the value of FeBIO (Table 1.5). Overall, based on these peak SNP markers for the traits evaluated the most favorable allele combination was a TT for marker S6_26904824 associated with protein concentration, GG for variant S7_80695 associated with Zn accumulation, and CC for SNP S11_1331783 associated with FeBIO. These peak SNPs had a positive effect on the phenotypes based on the association analyses and also these traits were positively correlated to each other, which are both desired scenarios for successful conventional and molecular breeding of the aforementioned phenotypes.

Candidate genes associated with the significant SNPs for the evaluated traits

In this study, we found candidate genes relevant to seed Zn and Ca homeostasis as well as FeBIO. One candidate gene was found from this work that is relevant to Zn homeostasis in common bean. Zinc homeostasis in plants requires both molecular and physiological processes that include uptake by the root system, distribution to source tissues, remobilization and redistribution from those tissues, and final loading or accumulation to various sinks throughout the plant, such as seeds (Bouain et al. 2014). The broader activities for genes involved in Zn homeostasis include Zn ion binding, catalytic activities by some enzymes, transcription regulator and cation diffusion or transporter activities (Broadley et al. 2007). After browsing the *Phaseolus vulgaris,* genome we detected a locus for seed Zn concentration *Phvul.010G023900* on Pv10. This

candidate gene was tagged by the SNP variant S10_22707473 that had an R^2 value of 7.18 % as the phenotypic variance explained in seed Zn concentration. This locus encodes for a heavy metal ATPase gene and was found at 0.66 Mb downstream of the candidate SNP. Heavy metal ATPase class of genes has been reported to play critical roles in translocation of several metal ions including Zn (Williams and Mills 2005). Functional studies in *Arabidopsis thaliana* and rice (*Oryza sativa* L.) have indicated that heavy metal ATPases are involved in Zn accumulation and distribution to different tissues including storage organs like seeds. For instance, a null mutant in *Arabidopsis thaliana* for *AtHMA4* had higher levels of Zn in the roots but less in shoot tissues, which indicated that *AtHMA4* might be involved in the loading of Zn to the xylem and subsequent trafficking to the above ground tissues (Hussain et al. 2004; Verret et al. 2004). Similarly, in rice, two genes *OsHMA1* and *OsHMA2* have been functionally characterized to be involved in the accumulation and transport of Zn ions from roots to shoots (Takahashi et al. 2012a). Takahashi et al. (2012b) demonstrated that rice genotypes with suppressed *OsHMA2* accumulated less Zn concentration in the leaves than in the root tissues when compared to the wild type, suggesting that this gene is involved in the accumulation of Zn in the roots and subsequent translocation from the root to the shoot tissues. In a more recent study, Astudillo-Reyes et al. (2015) reported a transcriptome profile of two bean genotypes (Albion and Voyager) highly contrasting for Zn accumulation phenotypes. Several expressed genes involved in Zn accumulation were reported and the candidate gene (*Phvul.010G023900:* Heavy metal ATPase) uncovered by GWAS from our study was also consistent with their findings. This candidate gene and SNP markers could be targeted for Zn improvement in the seed of common bean through use of marker-assisted selection and breeding.

Three loci for seed Ca concentration that encoded for calmodulin proteins were identified on Pv01. The SNP on Pv01 (S1_48843183) tagged locus *Phvul.001G226000* found at 0.77 Mb upstream of the marker. The same genetic marker also tagged loci *Phvul.001G226100* and *Phvul.001G226200* which were both located at 0.76 Mb upstream of the candidate SNP. This SNP explained about 8.07% of the variability in Ca concentration in the cooked bean seed samples and had a minor allele frequency of 0.06 (Table 1.5). Calmodulins are key sensors of calcium concentrations in plant tissues and by binding with Ca ions, they facilitate the subsequent translocation of Ca across cell membranes (Perochon et al. 2011).

Results of the GWAS for iron bioavailability, indicated a peak SNP on Pv11 (S11_1331783) that had a MAF of 0.07 and explained 13.2 % of the variability in FeBIO. This SNP also tagged a candidate gene *Phvul.011G020200* that encodes for 4-coumarate: CoA ligase 3. This locus was found at 0.25 Mb downstream of the candidate SNP. This *Phvul.011G020200* locus had a homologue in Arabidopsis (*AT3G21230*) that encodes for 4-coumarate:CoA ligase 5 based on the top hit in the BLASTN results in TAIR (*E* value = 2×10^{-7}). In phytozome, this gene was described as being involved in flavonoid biosynthesis, which is an important component of the phenylpropanoid pathway in plants. Flavonoids are the largest class of polyphenolic compounds in plants and some members of this class are responsible for differences observed in the pigmentation of leaves, flowers, and seeds (Manach et al. 2004). Moreover, there is growing evidence in common bean that certain polyphenolic compounds can have inhibitory while others have promoter effects on FeBIO based on the Caco-2 cell *in vitro* assay (Hart et al. 2015 and 2017; Hu et al. 2006; Tako et al. 2014). Given that this candidate gene is involved in the biosynthesis of flavonoids in both common bean and Arabidopsis, it should be explored in future experiments for its role in FeBIO and potential deployment in marker-assisted selection.

Conclusion

The genome-wide approach used in this study enabled a better resolution of the location of candidate markers and genes that should facilitate the improvement of common bean for nutritional quality, based on cooked samples. This is the first report of efforts to conduct GWAS for nutritional quality traits in common bean, particularly those of the Andean gene pool that is widely grown and consumed in sub-Saharan Africa. We detected QTL and candidate genes for most of the evaluated traits. These marker-trait associations need to be validated in other mapping populations as well as in the Mesoamerican genepool to determine concordance of our findings with future efforts. One important observation is that while all of our phenotypic evaluations were conducted on cooked seed samples the protein, Zn, and Fe concentration values were comparable to those reported in previous studies based on raw bean samples. Also, the consistent positive correlations among seed protein, Zn, FeBIO, and K concentrations is encouraging since this can potentially allow the simultaneous selection for these traits to be conducted successfully in a common bean nutritional quality breeding program.

Acknowledgements

This work was supported in part by funding from the Norman Borlaug Commemorative Research Initiative (U.S Agency for International Development), and by the U.S Department of Agriculture, Agricultural Research Service. The contents of this publication do not necessarily reflect the views or policies of the U.S Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S Government.

Table 1.1: Phenotypic summary statistics of the nutritional traits in cooked seed samples for all the 206 common bean genotypes grown in Michigan, USA for 2012 and 2013 field seasons.

Traits	Mean (± SD)	Minimum	Maximum	Fold-variation
Protein (%)	24.5 (1.5)	20.4	27.5	1.4
Zn (µg g^{-1})	31.8 (3.1)	21.6	39.7	1.8
Fe (µg g^{-1})	73.5 (7.3)	54.5	99.3	1.8
FeBIO (% of navy bean control)	48.2 (16.9)	20.4	110.7	5.4
K (mg g^{-1})	8.7 (0.9)	5.6	11.1	2.0
Ca (mg g^{-1})	1.2 (0.2)	0.7	2.1	2.9
P (mg g^{-1})	4.5 (0.4)	3.2	5.7	1.8
Phytic acid (mg g^{-1})	17.2 (1.5)	12.6	20.6	1.6

± *SD* refers to the standard deviation of the trait means; *Fold variation* refers to the maximum value relative to the minimum value for each trait

Table 1.2: ANOVA showing mean squares and percentage of total variance explained for seed protein, zinc, iron, and iron bioavailability of 206 common bean genotypes evaluated for two field seasons at Montcalm research farm in Michigan, USA.

		Traits							
		Protein (%)		Zn (µg g^{-1})		Fe (µg g^{-1})		FeBIO (% of navy bean)	
Source of variation	DF	Mean square	Percentage of TSS Explained	Mean square	Percentage of TSS Explained	Mean square	Percentage of TSS Explained	Mean square	Percentage of TSS Explained
Genotype	205	8.8***	54.1	39.7***	58.9	214.1***	50.2	1146.9***	40.2
Year	1	18.1**	0.5	263.8***	1.9	3349.0***	3.8	34035.5***	5.8
Genotype x Year	205	3.1***	19.2	11.4***	16.9	69.3 NS	16.2	645.2***	22.6
Rep(Year)	2	48.0***	2.9	68.0***	1.0	1293.3***	3.0	1116.9 NS	0.4

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 1.3: ANOVA showing mean squares and percentage of total variance explained for seed potassium, calcium, phosphorus, and phytic acid of 206 common bean genotypes evaluated for two field seasons at Montcalm research farm in Michigan, USA.

		Traits							
		K (mg g^{-1})		Ca (mg g^{-1})		P (mg g^{-1})		Phytic Acid (mg g^{-1})	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained
Genotype	205	3.5***	36.1	0.2***	56.1	0.7***	58.5	8.8***	23.6
Year	1	810.7***	40.2	12.2***	14.2	10.6***	4.2	4166.6***	54.4
Genotype x Year	205	1.1***	11.4	0.1***	12.5	0.2*	14.5	3.5***	9.5
Rep(Year)	2	0.9 NS	0.1	0.4***	0.9	1.3***	1.0	12.5*	0.3

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 1.4: Pearson correlation coefficients among nutritional traits for all the 206 common bean genotypes grown in Michigan, USA for the 2012 and 2013 field seasons

Trait	Zn	Fe	FeBIO	K	Ca	P	Phytic Acid
Protein	0.50***	0.50***	0.20**	0.45***	-0.20**	0.58***	0.40***
Zn		0.59***	0.01 NS	0.32***	0.01 NS	0.60***	0.45***
Fe			0.17 NS	0.29***	-0.10 NS	0.45***	0.33***
FeBIO				0.41***	-0.06 NS	0.24***	0.26***
K					-0.12 NS	0.76***	0.54***
Ca						0.01 NS	0.28***
P							0.74***

* Significance at the 0.05 probability level. ** Significance at the 0.01 probability level. *** Significance at the 0.001 probability level. NS, not significant

Table 1.5: Details of the top five loci that were significantly associated with the nutritional traits identified using MLM-based genome-wide association study across the 206 common bean genotypes grown in Michigan, USA for the 2012 and 2013 field seasons

Trait	Chr.	SNP	Position (bp)	Major Allele	Minor Allele	MAF	*P* value	R^2 (%)	Candidate gene : Annotation
Protein	Pv06	S6_26904824	26,904,824	C	T	0.07	1.46E-05	11.6	-
Protein	Pv07	S7_7597332	7,597,332	A	G	0.25	4.30E-04	6.3	-
Protein	Pv03	S3_43953342	43,953,342	G	A	0.41	4.41E-04	7.9	-
Protein	Pv06	S6_27577671	27,577,671	C	A	0.06	5.28E-04	7.7	-
Protein	Pv03	S3_43953289	43,953,289	T	A	0.44	7.31E-04	7.4	-
Zn	Pv07	S7_80695	80,695	G	A	0.19	1.45E-05	9.5	-
Zn	Pv07	S7_338865	338,865	T	C	0.37	8.94E-05	9.5	-
Zn	Pv07	S7_338867	338,867	C	G	0.37	8.94E-05	9.5	-
Zn	Pv07	S7_338868	338,868	C	T	0.36	1.57E-04	8.9	-
Zn	Pv07	S7_295275	295,275	G	A	0.23	1.87E-04	10.4	-
Zn	Pv10	S10_2707473	2,707,473	A	G	0.20	7.72E-04	7.2	*Phvul.010G023900* : Heavy metalATPase
Ca	Pv11	S11_7613114	7,613,114	A	C	0.31	7.41E-05	9.8	-
Ca	Pv02	S2_14889467	14,889,467	G	T	0.08	2.18E-04	8.6	-
Ca	Pv02	S2_28089586	28,089,586	C	T	0.06	3.24E-04	8.3	-
Ca	Pv04	S4_47502132	47,502,132	A	G	0.25	3.32E-04	8.2	-

Table 1.5 (cont'd)

Ca	Pv01	S1_48843183	48,843,183	T	C	0.06	3.76E-04	8.1	*Phvul.001G226000* Calmodulin
FeBIO	Pv11	S11_1331783	1,331,783	C	G	0.07	2.34E-05	13.2	*Phvul.011G020200* : 4CL3
FeBIO	Pv06	S6_28278333	28,278,333	G	T	0.48	5.71E-05	9.8	-
FeBIO	Pv06	S6_23708741	23,708,741	T	C	0.05	2.30E-04	8.4	-
FeBIO	Pv07	S7_36385041	36,385,041	A	G	0.33	2.40E-04	8.3	-
FeBIO	Pv06	S6_27783532	27,783,532	C	T	0.36	2.50E-04	8.8	-

Chr. is the *Phaseolus vulgaris* chromosome; *SNP* refers to single nucleotide polymorphic marker; *MAF* is the minor allele frequency; R^2 is the proportion of variability explained by the significant SNP for a particular marker-trait association test; 4CL3 is 4-coumarate: CoA ligase 3 gene.

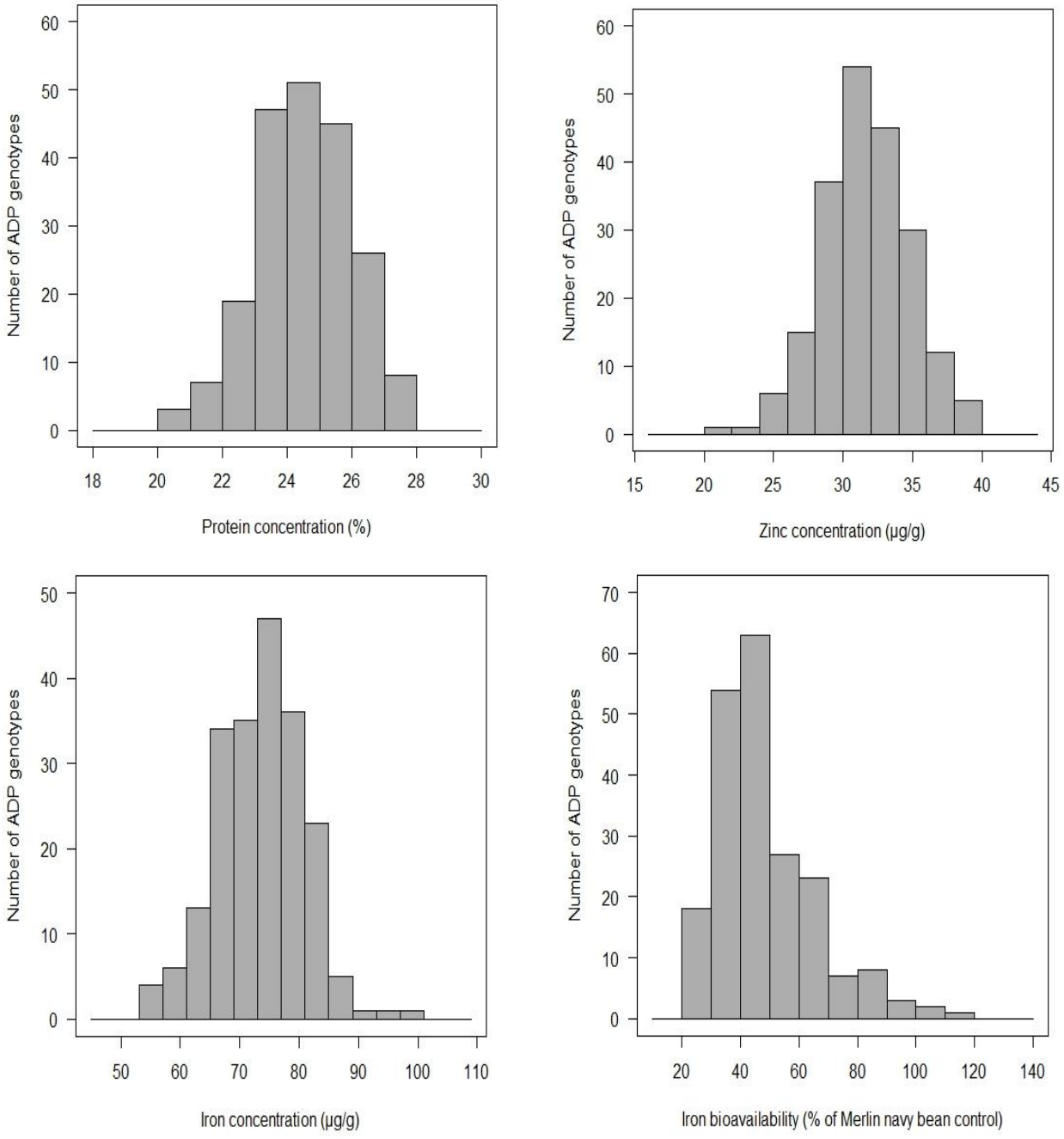


Figure 1.1: Histogram showing the distribution of seed protein, zinc, iron, and iron bioavailability in the cooked bean samples

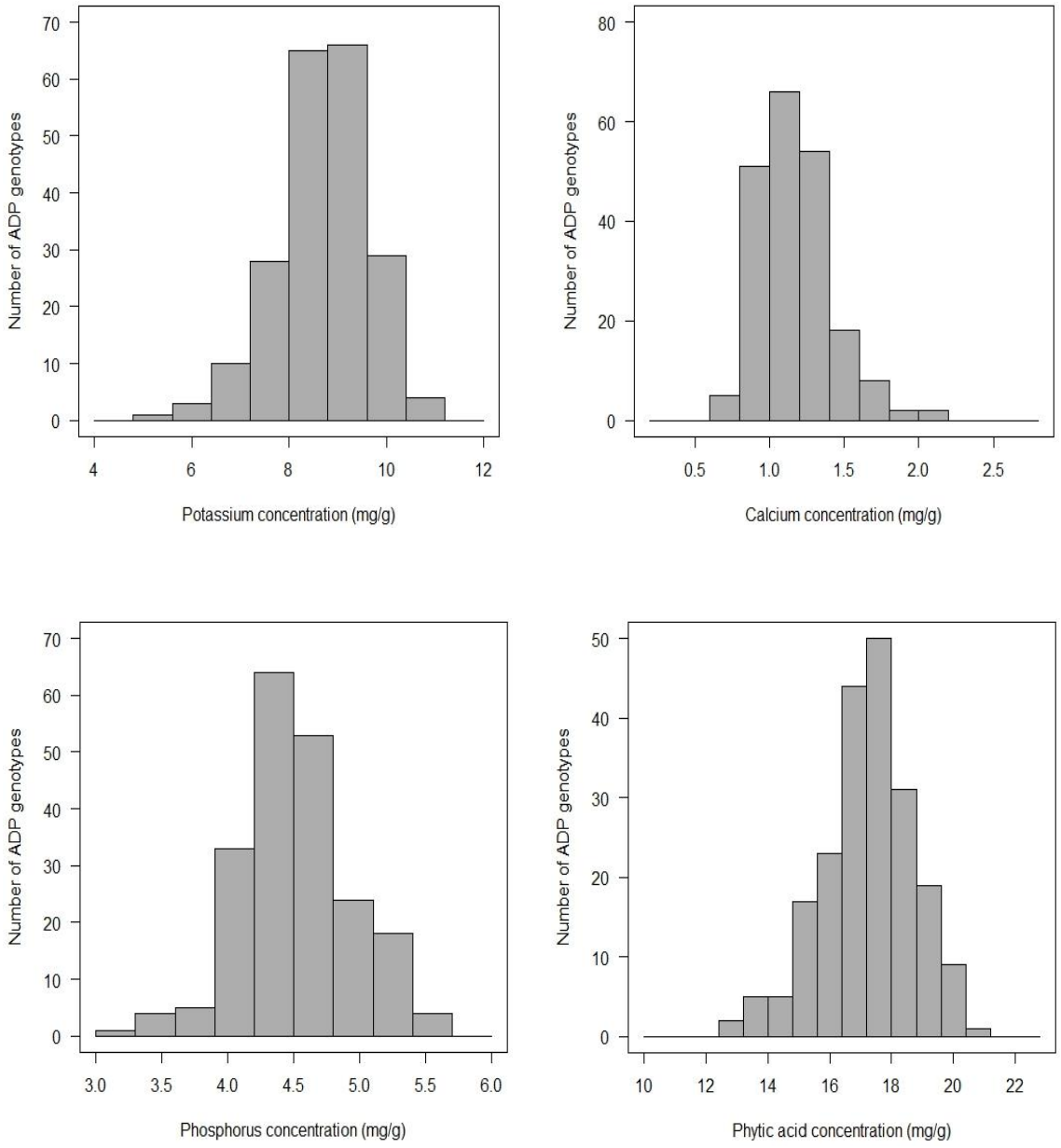


Figure 1.2: Histogram showing the distribution of seed potassium, calcium, phosphorus, and phytic acid concentration in the cooked bean samples

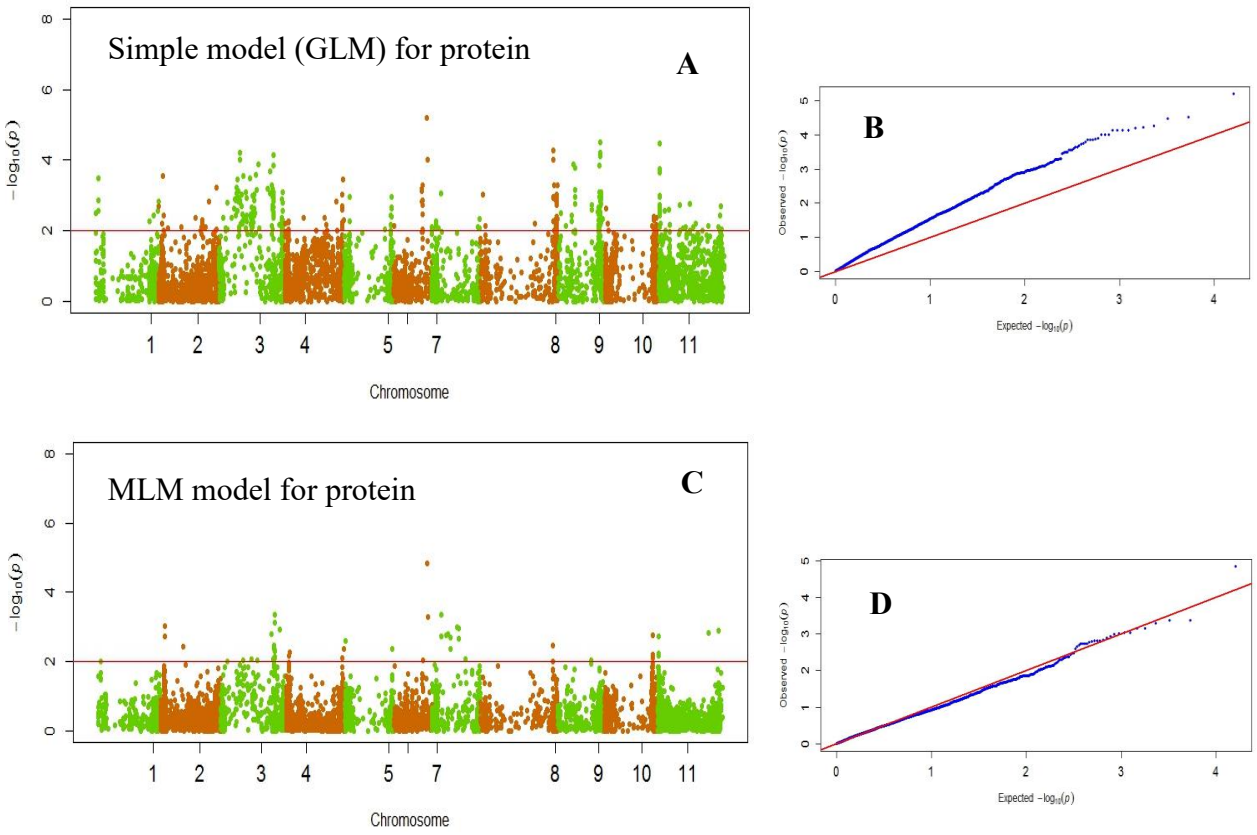


Figure 1.3: Manhattan plots depicting genome-wide marker-trait association for seed protein concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated

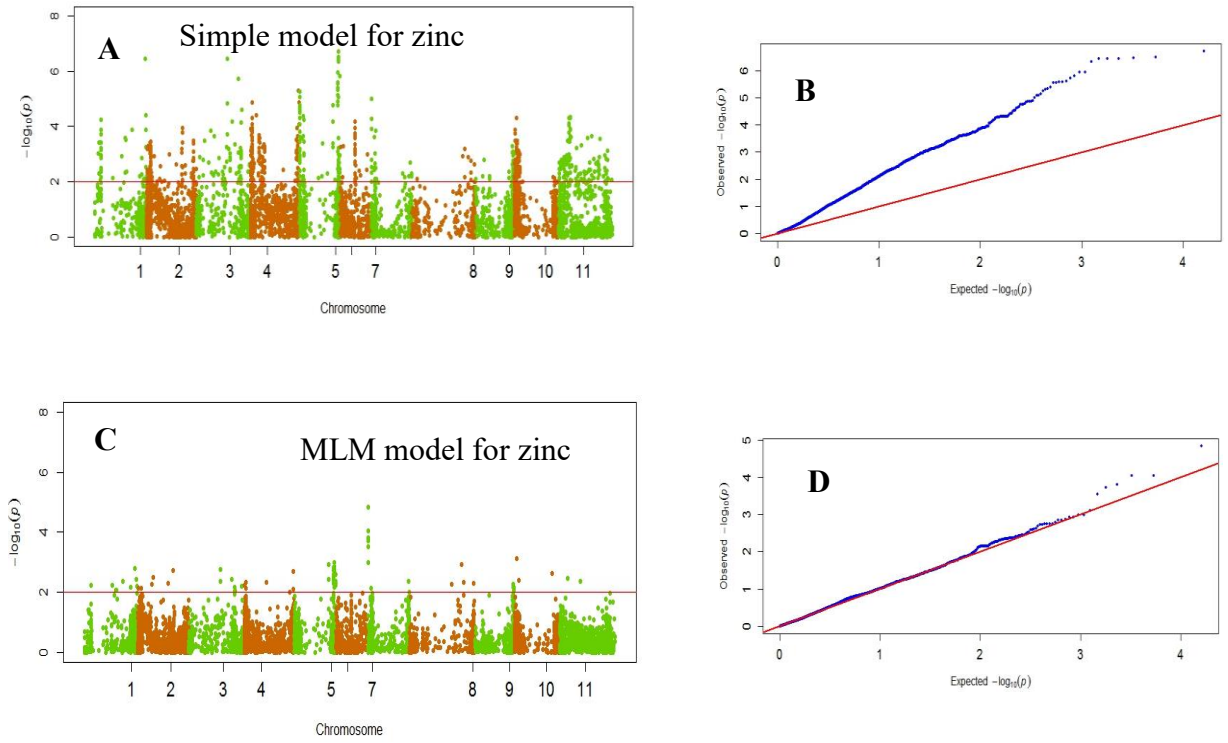


Figure 1.4: Manhattan plots depicting genome-wide marker-trait association for seed zinc concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated

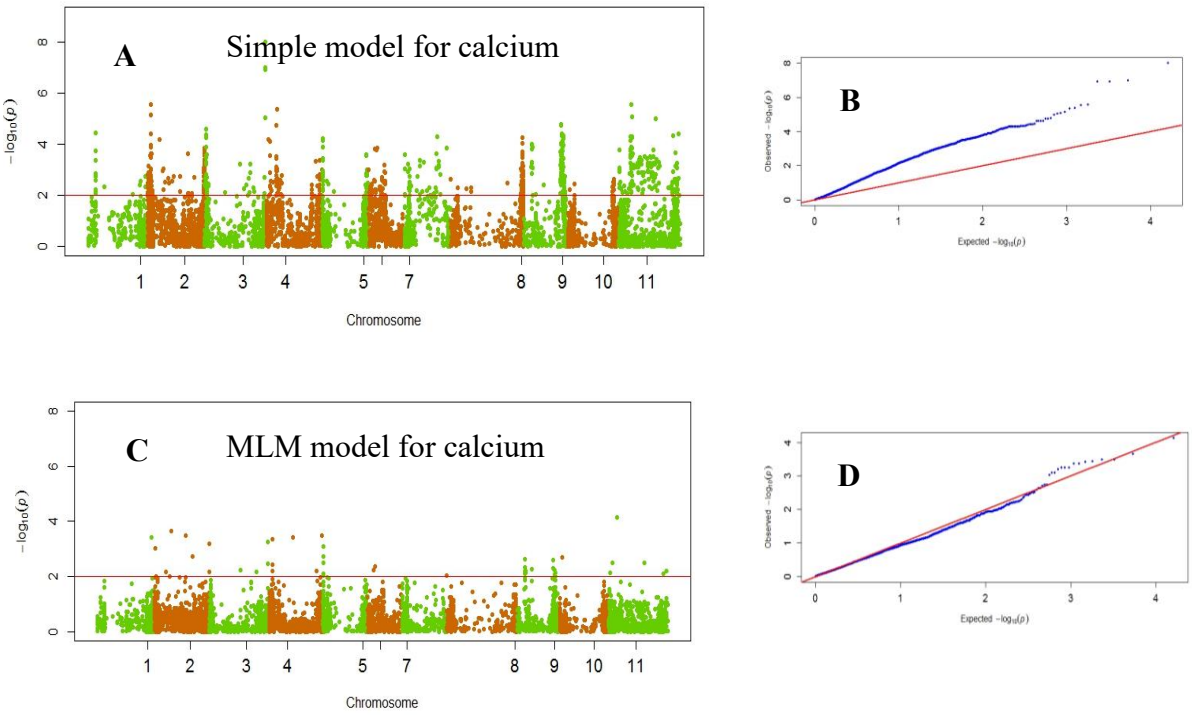


Figure 1.5: Manhattan plots depicting genome-wide marker-trait association for seed calcium concentration for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated

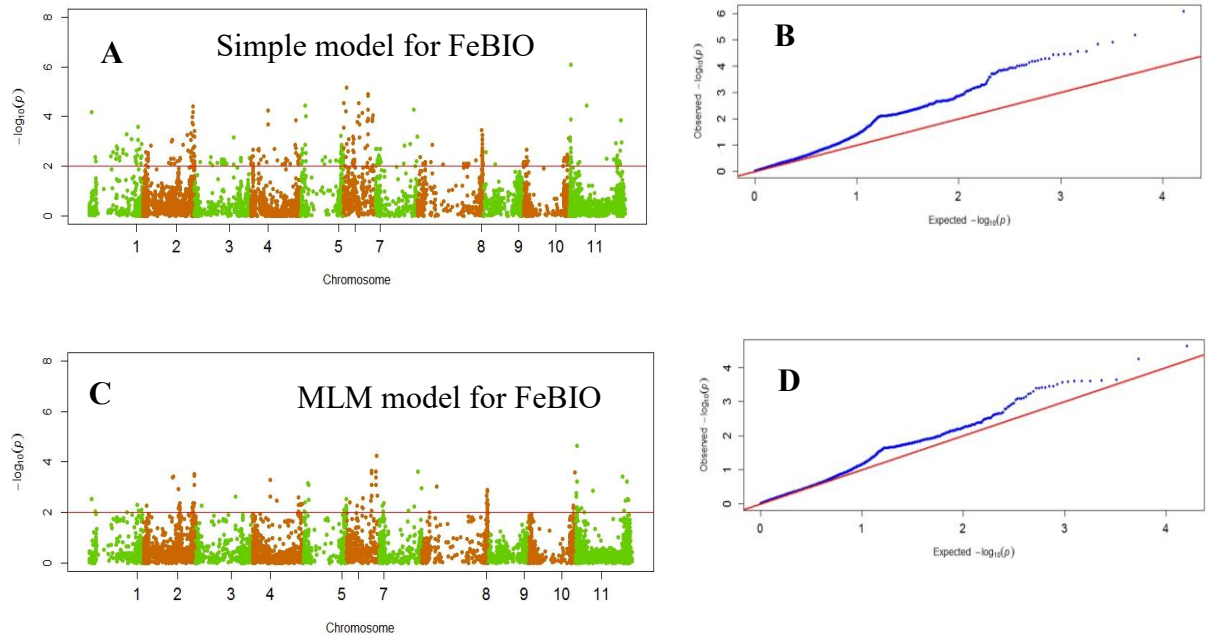


Figure 1.6: Manhattan plots depicting genome-wide marker-trait association for seed FeBIO for GLM (A) and MLM (C). The cutoff (red horizontal line) is based on the *q*FDR ≤ 0.1 used to declare genome-wide significance. The Quantile-Quantile plots testing for model goodness of fit and SNP marker inflation for GLM (B) and MLM (D) are also indicated

LITERATURE CITED

LITERATURE CITED

Akibode CS, Maredia M (2011) Global and regional trends in production, trade and consumption of food legume crops. Report submitted to the Standing Panel on Impact Assessment (SPIA) of the CGIAR Science Council, FAO, Rome

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389-3402

AOAC (1980) Official methods of analysis, 11th ed. Association of Official Analytical, Chemists, Washington, D.C.

Asif M, Rooney LW, Ali R, Riaz MN (2013) Application and opportunities of pulses in the food system: A review. Crit Rev Food Sci Nutr 53:1168-1179. doi:10.1080/10408398.2011.574804

Astudillo-Reyes A, Fernandez AC, Cichy KA (2015) Transcriptome characterization of developing bean (*Phaseolus vulgaris* L.) pods from two genotypes with contrasting seed zinc concentrations. PLoS ONE 10:e0137157. doi: 10.1371/journal.pone.0137157

Bass JK, Chan GM (2006) Calcium nutrition and metabolism during infancy. Nutr 22:1057-1066

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J of Stat Software, 67:1-48. doi:10.18637/jss.v067.i01

Beebe SE (2012) Common bean breeding in the tropics. Plant Breed. Rev 36:357-426. doi: 10.1002/9781118358566.ch5

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. (2008) Maternal and child undernutrition: Global and regional exposures and health consequences. Lancet 371:243-260. doi:10.1016/s0140-6736(07)61690-0

Blair MW, Izquierdo P (2012) Use of the advanced backcross-QTL method to transfer seed mineral accumulation nutrition traits from wild to Andean cultivated common beans. Theor Appl Genet 125:1015-1031. doi:10.1007/s00122-012-1891-x

Blair MW, Herrera AL, Sandoval TA, Caldas GV, Filleppi M, Sparvoli F (2012) Inheritance of seed phytate and phosphorus levels in common bean (*Phaseolus vulgaris* L.) and association with newly-mapped candidate genes. Mol Breed 30:1265-1277.

doi:10.1007/s11032-012-9713-z

Blair MW, Astudillo C, Rengifo J, Beebe SE, Graham R (2011) QTL analyses for seed iron and zinc concentrations in an intra-genepool population of Andean common beans (*Phaseolus vulgaris* L.). Theor Appl Genet 122:511-521. doi:10.1007/s00122-010-1465-8

Blair MW, Astudillo C, Grusak MA, Graham R, Beebe SE (2009a) Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.). Mol Breed 23:197-207. doi:10.1007/s11032-008-9225-z

Blair MW, Medina JI, Astudillo C, Rengifo J, Beebe SE, Machado G, et al. (2010a) QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (*Phaseolus vulgaris* L.) population. Theor Appl. Genet 121:1059-1070. doi:10.1007/s00122-010-1371-0

Blair MW, Gonzalez LF, Kimani PM, Butare L (2010b) Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. Theor Appl Genet 121:237-248. doi:10.1007/s00122-010-1305-x

Blair MW, Sandoval TA, Caldas GV, Beebe SE, Paez MI (2009b) Quantitative trait locus analysis of seed phosphorus and seed phytate content in a recombinant inbred line population of common bean. Crop Sci 49:237-246. doi:10.2135/cropsci2008.05.0246

Blair MW, Wu X, Bhandari D, Astudillo C. (2016) Genetic dissection of ICP-detected nutrient accumulation in the whole seed of common bean (*Phaseolus vulgaris* L.). Front Plant Sci 7:219-217. doi:10.3389/fpls.2016.00219

Boccio JR, Iyengar V (2003) Iron deficiency: Causes, consequences, and strategies to overcome this nutritional problem. Biol Trace Elem Res 94:1-32

Bouain N, Shahzad Z, Rouached A, Khan GA, Berthomieu P, Abdelly C, et al. (2014) Phosphate and zinc transport and signalling in plants : Towards a better understanding of their homeostasis interaction. J Exp Bot 65:5725-5741. doi:10.1093/jxb/eru314

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635 doi:10.1093/bioinformatics/btm308

Bremner JM (1965) Total nitrogen. p. 1149-1178. In C.A. Black et al (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Brinch-Pedersen H, Borg S, Tauris B, Holm PB (2007) Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. J Cereal Sci 46:308-326. doi:10.1016/j.jcs.2007.02.004

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677-

702. doi:10.1111/j.1469-8137.2007.01996.x

Caballero B (2002) Global patterns of child health: The role of nutrition. Ann Nutr Metab 46:3-7

Campion B, Sparvoli F, Doria E, Tagliabue G, Galasso I, Fileppi M, et al. (2009) Isolation and characterisation of an lpa (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 118:1211-1221. doi:10.1007/s00122-009-0975-8

Campion B, Glahn RP, Tava A, Perrone D, Doria E, Sparvoli F, et al. (2013) Genetic reduction of antinutrients in common bean (*Phaseolus vulgaris* L.) seed, increases nutrients and *invitro* iron bioavailability without depressing main agronomic traits. Field Crops Res 141: 27-37. doi:10.1016/j.fcr.2012.10.015

Casanas F, Perez-Vega E, Almirall A, Plans M, Sabate J, Ferreira JJ (2013) Mapping of QTL associated with seed chemical content in a RIL population of common bean (*Phaseolus vulgaris* L.). Euphytica 192:279-288. doi:10.1007/s10681-013-0880-8

Cichy KA, Porch TG, Beaver JS, Cregan P, Fourie D, Glahn RP, Grusak MA, Kamfwa K, Katuuramu DN, McClean P, Mndolwa E, Nchimbi-Msolla S, Pastor-Corrales MA, Miklas PN (2015a) A *Phaseolus vulgaris* Diversity Panel for Andean Bean Improvement. Crop Sci 55:2149-2160. doi:10.2135/cropsci2014.09.0653

Cichy KA, Wiesinger JA, Mendoza FA (2015b) Genetic diversity and genome-wide association analysis of cooking time in dry bean (*Phaseolus vulgaris* L.). Theor Appl Genet 128:1555-1567. doi:10.1007/s00122-015-2531-z

Cichy KA, Caldas GV, Snapp SS, Blair MW (2009a) QTL analysis of seed iron, zinc, and phosphorus levels in an Andean bean population. Crop Sci 49:1742-1750. doi:10.2135/cropsci2008.10.0605

Cichy KA, Blair MW, Mendoza CHG, Snapp SS, Kelly JD (2009b) QTL analysis of root architecture traits and low phosphorus tolerance in an Andean bean population. Crop Sci 49:59-68. doi:10.2135/cropsci2008.03.0142

Darnton-Hill I, Webb P, Harvey PW, Hunt JM, Dalmiya N, Chopra M, Ball MJ, Bloem MW, de Benoist B (2005) Micronutrient deficiencies and gender: Social and economic costs. Amer J Clin Nutr 81:S1198-S1205

Delaney DE, Bliss FA (1991) Selection for increased percentage phaseolin in Common bean: 1. Comparison of selection for seed protein alleles and S1 family recurrent selection. Theor Appl Genet 81:301-305. doi:10.1007/bf00228667

Duranti M, Gius C (1997) Legume seeds: Protein content and nutritional value. Field Crops Res 53:31-45. doi:10.1016/s0378-4290(97)00021-x

Ekmekcioglu C, Elmadfa I, Meyer AL, Moeslinger T (2016) The role of dietary potassium in

hypertension and diabetes. J Physiol Biochem 72:93-106. doi:10.1007/s13105-015-0449-1

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high density species. PLoS ONE. 6:e19379. doi:10.1371/journal.pone.0019379

Endelman JB, Jannink JL (2012) Shrinkage estimation of the realized relationship matrix. G3:Genes|Genomes|Genetics 2:1405-1413. doi:10.1534/g3.112.004259

Farnham MW, Keinath AP, Grusak MA (2011) Mineral concentration of Broccoli florets in relation to year of cultivar release. Crop Sci 51:2721-2727. doi:10.2135/cropsci2010.09.0556

Fileppi M, Galasso I, Tagliabue G, Daminati MG, Campion B, Doria E, Sparvoli F (2010) Characterization of structural genes involved in phytic acid biosynthesis in common bean (*Phaseolus vulgaris* L.). Mol Breed 25:453-470. doi: 10.1007/s11032-009-9344-1

Gao Y, Shang C, Maroof MAS, Biyashev RM, Grabau EA, Kwanyuen P, et al. (2007) A modified colorimetric method for phytic acid analysis in soybean. Crop Sci 47:1797-1803. doi:10.2135/cropsci2007.03.0122

Gibson RS (2006) Zinc: The missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc 65:51-60. doi:10.1079/pns2005474

Glahn RP, Lee OA, Yeung A, Goldman MI, Miller DD (1998) Caco-2 cell ferritin formation predicts non-radiolabelled food iron availability in an *in vitro* digestion/ Caco-2 cell culture model. J Nutr 128:1555-1561.

Glaubitz JC, Casstevens TM, Liu F, Harriman J, Elshire RJ, Sun Q, Buckler ES (2014) TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS ONE. 9:e90346. doi:10.1371/journal.pone.0090346

Goodstein DM, Shu SQ, Howson R, Neupane R, Hayes RD, Fazo J, et al. (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40:D1178-D1186. doi:10.1093/nar/gkr944

Ghosh S, Suri D, Uauy R (2012) Assessment of protein adequacy in developing countries: quality matters. Brit J Nutr 108(S2):S77-S87

Guzman-Maldonado SH, Martinez O, Acosta-Gallegos JA, Guevara-Lara F, Paredes-Lopez O (2003) Putative quantitative trait loci for physical and chemical components of common bean. Crop Sci 43:1029-1035. doi:10.2135/cropsci2003.1029

Hart JJ, Elad T, Glahn RP (2017) Characterization of polyphenol effects on inhibition and promotion of iron uptake by Caco-2 cells. J Agric Food Chem 65:3285-3294.

doi:10.1021/acs.jafc.6b05755

Hart JJ, Elad T, Kochian LV, Glahn RP (2015) Identification of black bean (*Phaseolus vulgaris* L.) Polyphenols that inhibit and promote iron uptake by Caco-2 cells. J Agric Food Chem 63:5950-5956. doi:10.1021/acs.jafc.5b00531

Hart JP, Griffiths PD (2015) Genotyping-by-sequencing enabled mapping and marker development for the *By-2* potyvirus resistance allele in common bean. Plant Gen 8. doi:10.3835/plantgenome2014.09.0058

Hirschi KD (2009) Nutrient biofortification of food crops. Annu Rev Nutr 29:401-421

Houston MC (2011) The importance of potassium in managing hypertension. Curr Hypertens Rep 13:309-317. doi:10.1007/s11906-011-0197-8

Hu Y, Cheng Z, Heller LI, Krasnoff SB, Glahn RP, Welch RM (2006) Kaempferol in red and pinto bean (*Phaseolus vulgaris* L.) coats inhibits iron bioavailability using an in vitro digestion/ human Caco-2 cell model. 54:9254-9261.

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Herper JF, Cobbetta CS (2004) P-Type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. Plant Cell 16:1327-1339. doi: 10.1105/tpc.020487

Ibarra-Perez FJ, Ehdaie B, Waines JG (1997) Estimation of outcrossing rate in common bean. Crop Sci 37:60-65. doi:10.2135/cropsci1997.0011183X003700010009x

Ingvarsson PK, Street NR (2011) Assocaition genetics of complex traits in plants. New Phytol 189:909-922. doi:10.1111/j.1469-8137.2010.03593.x

Islam FMA, Basford KE, Jara C, Redden RJ, Beebe SE (2002a) Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genet Resour Crop Evol 49:285-293. doi:10.1023/a:1015510428026

Islam FMA, Basford KE, Redden RJ, Gonzalez AV, Kroonenberg PM, Beebe SE (2002b) Genetic variability in cultivated common bean beyond the two major gene pools. Genet Resour Crop Evol 49:271-283. doi:10.1023/a:1015567513005

Kamfwa K, Cichy KA, Kelly JD (2015) Genome-wide assocaition analysis of symbiotic nitorgen fixation in common bean. Theor Appl Genet 128:1999-2017. doi:10.1007/s00122-015-2562-5

Kwak M, Gepts P (2009) Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theor Appl Genet 118:979-992

Latta M, Eskin M (1980) A simple and rapid colorimetric method for phytate determination. J Agric Food Chem 28:1313-1315. doi:10.1021/jf60232a049

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754-1760. doi:10.1093/bioinformatics/btp324

Lott JNA, Ockenden I, Raboy V, Batten GD (2000) Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci Res 10:11-33. doi:10.1017/S0960258500000039

Ma Y, Bliss FA (1978) Seed proteins of common bean. Crop Sci 18:431-437

Manach C, Scalbert A, Morand C, Remsey C, Jimenez L (2004) Polyphenols: food sources and bioavailability. Amer J Clin Nutr 79:727-747.

Matsumoto T, Wu JZ, Kanamori H, Katayose Y, Fujisawa M, Namiki N, et al. (2005) The map-based sequence of the rice genome. Nature 436:793-800. doi:10.1038/nature03895

Messina V (2014) Nutritional and health benefits of dried beans. Amer J Clin Nutr 100:437S-442S. doi:10.3945/ajcn.113.071472

Miller R, Spiro A, Stanner S (2016) Micronutrient status and intake in the UK – where might we be in 10 years' time? Nutri Bulletin 41:14-41. doi:10.1111/nbu.12187

Millward DJ, Jackson AA (2004) Protein/energy ratios of current diets in developed and developing countries compared with a safe protein/energy ratio: Implications for recommended protein and amino acid intakes. Public Health Nutr 7:387-405. doi:10.1079/phn2003545

Mitchell DC, Lawrence FR, Hartman TJ, Curran JM (2009) Consumption of drybeans, peas, and lentils could improve diet quality in the U.S population. J Amer Diet Assoc 109:909-913. doi:10.1016/j.jada.2009.02.029

Moraghan JT, Grafton K (2001) Genetic diversity and mineral composition of common bean seed. J Sci Food Agric 81:404-408. doi:10.1002/1097-0010(200103)81:4<404::AID-JSFA822>3.0.CO;2-H

Mudryj AN, Yu N, Aukema HM (2014) Nutritional and health benefits of pulses. Appl Physiol Nutr Metab 39:1197-1204. doi:10.1139/apnm-2013-0557

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. In: Symposium on Food Fortification in developing countries. J Nutr 136:1064-1067

O'Rourke JA, Iniguez LP, Fu F, Bucciarelli B, Miller SS, Jackson SA, McClean PE, Li J, Dai X, Zhao PX, Hernandez G, Vance CP (2014) An RNA-Seq based gene expression atlas of the common bean. BMC Genomics 15:866-881

Panzeri D, Cassani E, Doria E, Tagliabue G, Forti L, Campion B, et al. (2011) A defective ABC transporter of the MRP family, responsible for the bean lpa1 mutation, affects the

regulation of the phytic acid pathway, reduces seed myo-inositol and alters ABA sensitivity. New Phytol 191:70-83. doi:10.1111/j.1469-8137.2011.03666.x

Perochon A, Aldon D, Galaud JP, Ranty B (2011) Calmodulin and calmodulin-like proteins in plant calcium signaling. Biochimie 93:2048-2053. doi:10.1016/j.biochi.2011.07.012

Petry N, Egli I, Gahutu JB, Tugirimana PL, Boy E, Hurrell R (2012) Stable iron isotope studies in Rwandese women indicate that the common bean has limited potential as a vehicle for iron biofortification. J Nutr. 142:492-497

Pinheiro C, Baeta JP, Pereira AM, Domingues H, Ricardo CP (2010) Diversity of seed mineral composition of Phaseolus vulgaris L. germplasm. J Food Compos Anal 23:319-325. doi: 10.1016/j.jfca.2010.01.005

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38:904-909. doi:10.1038/ng1847

Raboy V (2007) The ABCs of low-phytate crops. Nat Biotechnol 25:874-875. doi:10.1038/nbt0807-874

Raboy V, Dickinson DB, Below FE (1984) Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *Glycine soja*. Crop Sci 24:431-434. doi:10.2135/cropsci1984.0011183X002400030001x

Rhee SY, Beavis W, Berardini TZ, Chen GH, Dixon D, Doyle A, et al. (2003) The Arabidopsis Information Resource (TAIR): a model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community. Nucleic Acids Res 31:224-228. doi:10.1093/nar/gkg076

Rizzoli R, Boonen S, Brandi ML, Burlet N, Delmas P, Reginster JY (2008) The role of calcium and vitamin D in the management of osteoporosis. Bone 42:246-249. doi:10.1016/j.bone.2007.10.005

Rodgers A, Ezzati M, Hoorn SV, Lopez AD, Lin RB, Murray CJL, et al. (2004) Distribution of major health risks: Findings from the global burden of disease study. PloS Med 1:44-55. doi:10.1371/journal.pmed.0010027

SAS Institute., 2011. SAS Version 9.4 SAS Institute Inc., Cary, NC, USA.

Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, et al. (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707-713. doi:10.1038/ng.3008

Schmutz J, Cannon SB, Schlueter J, Ma JX, Mitros T, Nelson W, et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature 463:178-183. doi:10.1038/nature08670

Schröder S, Mamidi S, Lee R, et al. (2016) Optimization of genotyping by sequencing (GBS) data in common bean (Phaseolus vulgaris L.). Mol Breed 36:1-9

Song Q, Jia G, Hyten DL, Jenkins J, Hwang EY, Schroeder SG, Osorno JM, Schmutz J, Jackson SA, McClean PE, Cregan PB (2015) SNP Assay Development for linkage map construction, anchoring whole-genome sequence, and other genetic and genomic applications in common bean. G3: Genes| Genomes| Genet 5:2285–2290. doi:10.1534/g3.115.020594

Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335:133-154. doi:10.1007/s11104-009-0228-2

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. PNAS 100:9440–9445

Swarts K, Li H, Romero JA, Navarro D, Romay MC, Hearne S, et al. (2014) Novel methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. Plant Gen 7:3-14. doi:10.3835/plantgenome2014.05.0023

Takahashi R, Bashir K, Ishimaru Y, Nishizawa NK, Nakanishi H (2012a) The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. Plant Signal Behav 7:1605-1607. doi: 10.4161/psb.22454

Takahashi R, Ishimaru Y, Shimo H, Ogo Y, Senoura T, Nishizawa NK, Nakanishi H (2012b). The *OsHMA2* transporter is involved in root-to-shoot translocation of Zn and Cd in rice. Plant Cell Environ. 35:1948-1957. doi:10.1111/j.1365-3040.2012.02527.x

Tako E, Beebe SE, Reed S, Hart JJ, Glahn RP (2014) Polyphenolic compounds appear to limit the nutritional benefit of biofortified higher iron black bean (*Phaseolus vulgaris* L.). J Nutr. 13:28-37. doi:10.1186/1475-2891-13-28

Tako E, Blair MW, Glahn RP (2011) Biofortified red mottled beans (*Phaseolus vulgaris* L.) in maize and bean diet provide more bioavailable iron than standard red mottled beans: Studies in poultry (*Gallus gallus*) and an *in vitro* digestion/ Caco-2 model. Nutr J. 10:113-123. doi:10.1186/1475-2891-10-113

Tako E, Glahn RP (2011) White beans provide more bioavailable iron than red beans: Studies in poultry (*Gallus gallus*) and an in vitro digestion/ Caco-2 model. Int J Vitam Nutr Res. 81:1-14. doi:10.1024/0300-9831/a000028

Turner SD (2014) Qqman: An R package for visualizing GWAS results using Q-Q and Manhattan plots. http:/biorxiv.org.

U.S. Department of Agriculture and U.S. Department of Health and Human Services (2011) Report of the dietary guidelines for Americans. 7th edition. Washington, D.C: U.S. Government

Printing Office.

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P (2004) Over-expression of *AtHMA4* enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576:306-312. doi:10.1016/j.febslet.2004.09.023

Wang N, Daun JK (2005) Determination of cooking times of pulses using an automated Mattson cooker apparatus. J Sci Food Agric 85:1631-1635

Weaver CM (2013) Potassium and Health. Adv Nutr 4:368S-377S. doi:10.3945/an.112.003533

White PJ (2013) Improving potassium acquisition and utilization by crop plants. J Plant Nutr Soil Sci 176:305-316. doi:10.1002/jpln.201200121

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92:487-511. doi:10.1093/aob/mcg/164

Williams LE, Mills RF (2005). P(1B)-ATPases – An ancient family of transition metal pumps with diverse functions in plants. Trends Plant Sci 10:491-502. doi:10.1016/j.tplants.2005.08.008

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203-208. doi:10.1038/ng1702

Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, et al. (2010) Mixed linear model approach adapted for genome-wide association studies. Nat Genet 42:355-360. doi:10.1038/ng.546

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Gen 1: 5-20. doi:10.3835/plantgenome2008.02.0089

Zimmermann MB, Hurrell RF (2007) Nutritional iron deficiency. Lancet. 370:511-520

CHAPTER 2

EVALUATION OF GENOTYPE BY ENVIRONMENT INTERACTIONS FOR AGRONOMIC, COOKING TIME, AND NUTRITIONAL QUALITY TRAITS IN COMMON BEAN ACCESSIONS GROWN ON-FARM IN UGANDA

Evaluation of genotype by environment interactions for agronomic, cooking time, and nutritional quality traits in common bean accessions grown on-farm in Uganda

Abstract

Dry bean (*Phaseolus vulgaris* L.) is a staple food and cash crop for millions of people around the world. It supplies protein, fiber, and minerals (macro and micronutrients) to consumers. Limited genotype by environment information is available for dry bean traits valued by consumers such as cooking time and nutritional value. This study evaluated fifteen dry bean test genotypes and a local check variety mostly within the red mottled, yellow and kidney market classes at nine on-farm locations in Uganda for two field seasons (2015 and 2016). Fourteen traits important to growers and consumers were evaluated, including seed yield, cooking time and cooked bean mineral concentrations. On farm yields ranged from 525 to 928 kg ha^{-1} and two of the consistently high yielding genotypes (Chijar and Amarelo Cela) were from the Middle American gene pool. Cooking time ranged from 24 to 96 minutes and the yellow beans Cebo Cela and Ervilha consistently cooked fastest in 24 and 27 minutes respectively. The genotype with the highest Fe concentration was a red mottled landrace Rozi Koko with an average seed Fe concentration of 75 µg g^{-1} across all locations and years. It also had the highest seed Zn concentrations of 32 µg g^{-1}. There was a positive correlation among seed Fe, Zn, and Mg concentration. The GGE biplot analysis uncovered presence of two mega-environments for seed yield and one mega-environment for cooking time andseed Fe and Zn concentrations. Identification of mega-environments for these traits in Uganda will help accelerate common bean variety breeding, evaluation, and selection by reducing the number of environments needed for phenotypic evaluation. The fast cooking and high mineral genotypes can be targeted for improving common bean for cooking quality and nutrient composition.

Introduction

With the increasing world population and a projection of 10 billion people by the year 2050, plant varieties that are productive and stable under changing biotic and abiotic threats are urgently needed (Alexandratos and Bruinsma 2012). Given the high levels of micronutrient malnutrition (Bailey et al., 2015), productive and nutritious common bean varieties and germplasm need to be identified for crop improvement and release to target communities. Such germplasm once identified can also be deployed in breeding programs for countries that need them the most. Plants can accumulate micronutrients such as iron and zinc as well as macronutrients such as calcium, potassium, phosphorus, and magnesium into the edible plant parts like leaves and seeds (Baxter et al., 2012). Through carefully designed plant hybridization and selection strategies, varieties with competitive yield and nutritional quality can be developed.

To make large gains from selection for both agronomic and consumer traits, plant breeders need to regularly determine the effects of genotype, environment, and genotype by environment interactions within the current sets of germplasm (Annicchiarico et al., 2005). Common bean growers demand high yielding varieties and consumers want nutrient-dense, fast cooking beans (Bucheyeki and Mmbaga, 2013). While seed yield across environments has been an important focus of bean breeding programs, less is known about the stability of end use traits of interest to consumers across environments and on farmers' fields. On-farm participatory evaluation of genotype and genotype x environment interactions has been used to efficiently identify novel germplasm well suited for particular agro-ecological conditions like for rice in India (Balakrishnan et al., 2016), sweet potatoes in Uganda and South Africa (Tumwegamire et al., 2016; Laurie et al., 2015), and cassava in Tanzania (Masinde et al., 2018). In common bean, genotype by environment studies have been restricted mostly to agronomic traits and response to some foliar diseases (Awio et al., 2017; Tokatlidis et al., 2010). There is limited information on multi-location studies for

cooking time and mineral nutrient composition in dry bean. On-farm variety trials have numerous advantages including direct feedback from the end users about the genetic materials as well as an opportunity to observe and measure genotype performances in an environment where they can potentially be released or used to improve the existing germplasm (Gyawali et al, 2007).

Common bean is a widely grown and consumed staple crop in many parts of Latin America and Africa including Uganda (Akibode and Maredia, 2012; Uganda Bureau of Statistics 2010). In Uganda, common bean is the most important legume crop (Sibiko et al., 2013). The crop can also thrive in a wide range of agro-ecological zones for instance, the small seeded black Middle-American beans can be productive in the drier parts of northern Uganda like Arua district while the bush type beans that are mostly red mottled, yellow, and cream colored perform well in parts of eastern, central, and western Uganda (Kiwuka et al., 2012; Okii et al., 2014). In the mountain regions with cooler climates like Kabale and Kisoro districts, its typical for farmers to grow the Type IV beans that are aggressive climbers usually with stakes or trellises and sometimes intercropped with maize for support (Okii et al., 2014). Given its wide range of adaptation and importance to growers as a food security and cash crop, it is vital to study the magnitude of genotype plus genotype x environment responses in common bean. The crop is also rich in a number of nutrients especially protein, fiber, iron, zinc, calcium, potassium, phosphorus, and magnesium (McClean et al., 2017). This makes common bean a desirable target for biofortification (mineral enhancement in the plant edible parts through plant breeding methods) (Blair, 2013; Webb and Kennedy, 2014). Micronutrient malnutrition especially iron and zinc affects a large portion of the Ugandan population (especially nursing mothers and children under five years old) as well as similar demographics from other developing countries in the world (Hotz et al., 2011; Gibson and Ferguson, 2008). Bean biofortification work has been on-going in Uganda and

HarvestPlus has targeted introduction of yellow, red mottled, and cranberry seed types for evaluation and release. Four genotypes developed through conventional plant breeding have been released to-date and two cultivars are yellow, while one is a red mottled and the other is a cranberry. There is growing information that for biofortified beans to be well adopted by farmers, they need to be high yielding and adapted to the changing weather patterns in the respective agro-ecological zones (Birol et al., 2015; Chowdhury et al., 2011). It is equally important to determine how the biofortified bean germplasm performs when grown in diverse environments on farmers'' field to identify parents. It is therefore important to extensively evaluate nutrient-dense germplasm for stable agronomic and micronutrient phenotypes since only such materials will be accepted and adopted by the growers.

Evaluation of genotype x environment and trait stability in response to different agro-ecological conditions for end use quality traits have not been adequately explored in common bean. We identified 15 genotypes with variability for cooking time and seed Fe and Zn concentration from a previous screening of an Andean diversity panel germplasm that was grown in Michigan (Cichy et al., 2015). Most of the yellow and white beans were fast cooking and had high Fe bioavailability. The red mottled genotypes were long cooking but high in seed Fe and Zn concentration. We therefore wanted to evaluate these 15 materials across three regions on-farm in Uganda to determine stability of the traits from our earlier studies as well as farmers' interest in the accessions. The three locations represent areas of high common bean consumption as well as marketing and selling. Kamuli and Hoima districts produce dry beans largely for home consumption while Masaka and Rakai produce beans as a food and cash crop (Kiwuka et al., 2012). The goals of this study were to estimate the magnitude of genotype and genotype x environment interactions at nine locations in Uganda for cooking time and nutritional quality phenotypes and

identify the best performing and stable genotypes for agronomic performance, cooking time, and nutritional quality phenotypes.

Materials and Methods

Field study sites

The study was conducted at nine on-farm locations representing three agro-ecological zones in Uganda. The agro-ecological zones include grassland savanna for Hoima district, tall savanna for Kamuli district, and tropical rainforest for both Masaka and Rakai districts. Three locations were in Hoima district in Western Uganda. The Hoima district locations included Kakindo (KA), Kyamaleera (KY), and Tugonzengane (TU) (Table 2.3 and Figure 2.1). Three location were in Kamuli district in Eastern Uganda and they included Katugezeeku (KU), Tweweeyo (TW), and Tweyunge (GE) (Table 2.3 and Figure 2.1). Both Masaka and Rakai districts are in Central Uganda (Figure 2.1). One location in Masaka district was Balitwewunya (BA) while the two locations of Agali Awamu (AG) and Kiyovu (KV) were in Rakai district (Table 2.3 and Figure 2.1). All study locations receive bimodal rainfall with the first rains falling from March to May while the second rainy season from mid-September to the end of early December. Both Masaka and Rakai districts are located near Lake Victoria in central Uganda, the districts receive annual rainfall ranging from 850 to 2,125 mm. The annual temperature varies from 15 to 27°C (Kimbugwe 2013; Mubiru et al., 2015). Hoima district in western Uganda receives rainfall ranging from 800 to 1,400 mm per year with temperature ranges of 15 to 30°C (Mubiru et al., 2015). Kamuli district is located in eastern Uganda and the area receives annual rainfall ranging from 800 to 1,300 mm with temperatures ranging from 16 to 31°C (Mubiru et al., 2015). Across all our study

locations variable amounts of rainfall, fluctuating number of rain days, and increasing changes in temperatures of 0.5 to 0.9°C have been reported (Mubiru et al., 2015).

Before planting, soil samples were collected from each site in both 2015 and 2016 seasons to be evaluated for soil nutrient composition at the Soil and Plant Nutrient Laboratory at Michigan State University, USA. The soils in Hoima were sandy clay, sandy clay loam, and clay for KA, KY, and TU respectively. The Kamuli soils were clay loam for site KU and sandy clay loam for both TW and GE locations. In Rakai district AG had sandy clay while KV had sandy clay loam soils. Location BA in Masaka district had clay soils (Table 2.3).

Common bean germplasm

The genotypes were chosen from a larger Andean Diversity Panel (ADP) germplasm screening of over 200 lines grown in Montcalm MI in 2012 and 2013 and characterized for cooking time, seed mineral concentration and iron bioavailability (Cichy et al., 2015). Genotypes targeted for inclusion into the 15-germplasm set had variable cooking times (from fast to slow cooking), Fe concentration greater than 70 µg g^{-1} and Zn concentration greater than 30 µg g^{-1} (Cichy et al., 2015). Three of the genotypes were dark red kidney, four were yellow, five were red mottled, one genotype for each of the white, light red kidney, and small red seed market classes. The local check NABE-15 (Kanyeebwa) is a cream mottled bean and was the most popular among farmers and was planted at six locations (KA, TU, KU, GE, KV, and BA) in both 2015 and 2016 field seasons. This variety was released in 2008 by Namulonge National Crops Resources Research Institute (NaCRRI) of Uganda, it's major attributes are high yield, fast cooking, and resistance to anthracnose. Locations KY and TW were planted with Masindi yellow local check which is an old landrace preferred for its taste and fast cooking. Location AG was planted with NABE-4 (Nambaale omuwanvu) local check also released by NaCRRI in 2000. NABE-4 has large red

mottled seed grain color, its fast cooking, drought tolerant, and high yielding. Eleven of the trial genotypes and all local checks were of determinate growth habit (Tables 2.1 and 2.2).

Experimental design

The experiment was conducted as a randomized complete block design with two field replications for all genotypes at the nine locations. Each genotype plot was comprised of five rows that were 3.5 m long with 0.5 m between row length. The entire five-row plot was planted with 220 dry bean seeds. The study was conducted for two field seasons during the long rainy season of 2015 September - through early December and the shorter rainy season of 2016 (March to May). Planting was initiated and completed at the onset of rains (first week of September in 2015) and (first week of March in 2016). A light application of starter fertilizer was applied at planting where triple 17 ratio of nitrogen, phosphorus, and potassium (NPK) was applied at a rate of 125 kg ha^{-1} to all sites in both seasons. The field trials were kept weed-free by two cycles of hand-weeding during the growing season in both 2015 and 2016, once at 30 and 45 days post-planting. Beans were harvested and threshed manually to collect agronomic data, cooking time, and then nutrient compositional analysis. Field plot harvesting was completed by the first week of December 2015 and third week of June 2016.

Phenotyping for bean foliar diseases and agronomic traits

We surveyed the fields at all the nine locations for incidences based on natural infections of the major bean foliar diseases in study locations. The diseases of interest were angular leaf spot (ALS) caused by *Pseudocercospora griseola*, common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli*, common bean rust (CBR) caused by *Uromyces appendiculatus*, bean common mosaic virus (BCMV) a member of the Potyvirus, and black root hypersensitive response

(BR) due to attack by the Bean common mosaic necrotic virus (BCMNV). We used the International Center for Tropical Agriculture (CIAT) disease incidence rating scale of 1-9 (Schoonhoven and Pastor-Corrales, 1987) for ALS, CBB, rust, and BCMV. On this scale a score of 1 indicates that the genotype plot is clean or has resistant plants and a score of 9 implies all the plants in a particular plot are dead and/or susceptible. Black root was evaluated by counting the number of plants showing signs of wilting and death per genotype plot. The 1-9 foliar disease rating scale developed by CIAT has been widely used to evaluate bean diseases in the field and under controlled environments (Schoonhoven and Pastor-Corrales, 1987). Seed yield was collected on all harvested plots and converted to kg/ha while 100-seed weight (100-SW) was collected by counting 100 seeds and determining their weight in grams.

Phenotyping for cooking time and mineral nutrient composition

A total of 25 seeds from each plot and location were cooked on a Mattson cooker as previously described in Cichy et al., 2015 to record cook times in minutes. Briefly, prior to cooking 25 seeds per genotype were soaked in deionized water for 12 hours. Cooking time was recorded when 80 % of the Mattson cooker pins dropped through the 25 seed samples per genotype. The cooked bean samples were then cooled, freeze-dried, and ground using a Spex Sample Prep 2000 Geno Grinder to pass through a 1 mm screen. About 20 g of bean powder was shipped to the Robert W. Holley Center for Agriculture and Health mineral analysis lab in Ithaca New York for mineral analysis. To quantify both macro and micro nutrients calibration standards were prepared and measurements performed using the inductively coupled plasma-mass spectrometer as described in Farnham et al., 2011.

Statistical analyses

The analysis of variance (ANOVA) for all sources of variation in the statistical model was conducted using the PROC GLM statement in the statistical analysis software, SAS v9.4, (SAS-Institute-Inc. 2011). Pearson correlation coefficients among traits across locations and years were determined using the PROC CORR statement in SAS. The variance components for estimating broad sense heritability (H^2) for all traits were computed using the PROC VARCOMP statement in SAS v9.4 using the restricted maximum likelihood method (SAS-Institute-Inc. 2011). The statistical model used ANOVA and computing variance components in SAS shown below:

$$Y = \mu + G + L + S + GxL + GxS + LxS + GxLxS + rep(LxS) + \varepsilon$$

Where Y is the response variable like seed yield or cooking time, μ is the grand mean, G is the effects of the evaluated common bean genotypes, L refers to the effects of locations, S is the effect of seasons, GxL is the interaction term between genotype and locations, GxS refers to the interaction term between genotype and seasons, LxS is the interaction between location and seasons, GxLxS is the interaction between genotype, location, and seasons, rep(LxS) denotes nesting of the replications within the location by season interaction, and ε is the error term. For the ANOVA in SAS, the effects of G, L, and GxL were treated as fixed while the remaining effects as random to estimate Fisher's protected least significance difference (LSD) and to compare means among the common bean genotypes and locations for each phenotype. To generate the variance components for computing broad-sense heritability estimates (H^2) all effects in the statistical model above were treated as random. Broad sense heritability (H^2) was computed using the equation below:

$$\text{Heritability} = \frac{\text{Var(G)}}{\text{Var(G)} + \frac{\text{Var(GxL)}}{\text{l}} + \frac{\text{Var(GxS)}}{\text{s}} + \frac{\text{Var(GxLxS)}}{\text{ls}} + \frac{\text{Var(Error)}}{\text{lsr}}}$$

where, Var(G) is genotypic variance, Var(GxL) is the genotype by location variance, Var(GxS) is the genotype by season variance, Var(GxLxS) is the genotype by location by season variance, Var(Error) is the residual/experimental error variance. The denominators l, s, and r represent number of locations, seasons, and field replications respectively.

To visually assess the presence of mega-environments, trait stability, and genotype rankings in our two-way data (Yan et al., 2000), a GGE biplot analysis was conducted using the GGEBiplotGUI package (Frustos et al., 2014) in RStudio. To generate two-way data required for GGE biplot analysis, all location by season combinations for every trait were defined as environments (Yan and Tinker, 2006) and the data files inputted into RStudio to visualize the GGE patterns. All of our data were tester centered (G+GE) and non-scaled as described in Yan and Tinker, 2006. Biplots intended to evaluate test environments and genotypes like "which-won-where" polygons were drawn with column metric preserving singular value partitioning (SVP). Biplots for evaluating genotype and genotype x environment main effects like mean performance vs. stability and genotype rankings were drawn with row metric preserving SVP (Yan, 2002).

Results

Soil chemistry characteristics

Over both years the soils at the test locations had pH ranging from 5.0 - 7.1 with locations in Rakai and Masaka districts (AG, KV, BA) having lower readings/ acidic soils (5.0 to 5.6) (Table 2.4). One site in Hoima district (KY) and another location GE in Kamuli had elevated pH levels over

both years. The soil organic matter was higher for 2015 field season at locations KA, KY, TW, and KV compared to that measured in 2015. Location GE had similar values of soil organic matter in both field seasons. The other four locations TU, KU, AG, and BA had higher soil organic matter in 2016 field season than those measured in 2015, which might be due to crop rotation systems that growers often use between maize, beans, sweet potatoes, and cassava on their small farm land holdings. Most locations except TW (2016), AG (2015), and KV 2016 had organic matter greater or equal to 3 %. (Table 2.4). All locations had lower soil nitrate concentration in the 2016 field season compared to readings observed in 2015 except for TW in Kamuli where the measurements were similar. Soil phosphorus ranged from 6 - 36 µg g^{-1} across all locations. Most sites except KY (2016) and BA (2015) had P levels below the optimum value of 36 µg g^{-1} required for good crop productivity on agricultural soils (Table 2.4). Soil K concentration ranged from 28 - 531 µg g^{-1} for the nine locations over the two field seasons. Six locations (KA, KY, KU, TW, GE, and BA) had higher soil K levels in the 2015 field season compared to the 2016 season. Locations TU, AG, and KV had lower soil K concentration in 2015 compared to the 2016 field season. Soil K levels of 144 µg g^{-1} are sufficient for crop growth and a number of locations didn't meet this soil K cutoff. Soil Zn concentration above 2.0 µg g^{-1} would be sufficient for crop growth and sites TU and GE had optimum levels in both 2015 and 2016 while the other locations had sufficient levels only in one field season. Soil Fe was sufficient for most locations except KA (2015) and TW (2015) (Table 2.4).

ANOVA, summary statistics, heritability, and trait correlations

Results of the analysis of variance (ANOVA) for all traits are presented in Table 2.4 to 2.8. Yield, seed Mg, CBB, and CBR were the only traits where significant effects were observed for all sources of variation in the ANOVA ($P \leq 0.05$). The variation in most of the traits studied was due genotypic and location effects (Tables 2.5 to 2.9).

Agronomic traits

The most important source of variation for yield was location (21.5 % of total sums of squares [TSS]) and the interaction component between location and season (48.6 % of TSS) (Table 2.5). 100-seed weight was largely controlled by genotype (51.5 % of TSS) followed by location x season interaction (19.9 % TSS) (Table 2.5). Seed yield and 100-seed weight (100-SW) had a fold change 1.8 considering the minimum and maximum values for these phenotypes (Table 2.10). Broad-sense heritability (H^2) estimates ranged from 53.3 to 96.5 %. Seed yield had a moderately high heritability estimate of 69.7 while 100-seed weight had a high broad-sense heritability estimate (Table 2.10).

End use quality

Bean cooking time was controlled to a large extent by genotype (40.6 % of TSS) followed by genotype x location (19.3 % of TSS) (Table 2.5). Cooking time had the highest fold variability of 3.9 among all the evaluated traits (Table 2.10). Cooking time had moderately high heritability estimates of 70.3 % (Table 2.10).

Seed nutrient density

The most important source of variation for seed Fe was genotype (27 % of TSS) followed by location (15 % of TSS) (Table 2.6). The ANOVA for seed Zn concentration showed that genotype and location were the major sources of variation for this trait as they accounted both for 23.1 % and 25.5 % of TSS respectively (Table 2.6). The variation in seed Ca was strongly controlled by genotype (41.8 % TSS) (Table 2.6). Seed K concentration was largely due genotype at 23.2 % of TSS followed by genotype x location (18.5 % of TSS) and location (16.3 % of TSS) (Table 2.7). In the case of seed P concentration, variation of 30.7 and 33.3 % of TSS was explained by genotype and location respectively. Seed Mg concentration was largely controlled by genotype (32.6 % of TSS), followed by location (13.7 % of TSS), and then location x season (13.1 % of TSS) (Table 2.7). Seed Fe, Zn, and Mg concentrations all had a fold change of 1.3 (Table 2.10). Seed Ca and K concentration both exhibited a fold variation of 1.7 while seed P had a fold variation of 1.4 across the 16 genotypes grown at nine locations for the two field seasons (Table 2.10). Seed Fe, Zn, Ca, K, P, and Mg concentrations had relatively high broad-sense heritability with values greater than 81 % (Table 2.10).

Disease scores

Location (24.3 % of TSS) explained most of the variation in severity of the ALS disease pressure observed in our study followed by genotype x location (15.3 % of TSS), and then genotype x location x season (11.9 % of TSS) (Table 2.8). Variation in CBB was largely controlled by genotype x location, location x season, and genotype x location x season based on the 17.1, 15.7, and 16.6 % of TSS values respectively (Table 2.8). As for CBR, both location and genotype x location had an identical control on most of the variation observed in our study (21 and 21.1 % of TSS respectively) (Table 2.8). For the BCMV bean foliar disease, most of the variation was

largely controlled by genotype, location, and genotype x location (22, 17.2, and 16 % of TSS respectively) (Table 2.9). Variation in presence of BR hypersensitive response in the field was largely explained by genotype x location and genotype (47 and 16.5 % of TSS respectively) as presented in the Table 2.9. BCMV incidences had a fold change 1.8 considering the minimum and maximum values for these phenotypes (Table 2.10). Severities of ALS and CBB in the field had a 1.4 fold variation across the 16 genotypes grown at nine locations for the two field seasons (Table 2.10). A 1.5 fold variability was observed for CBR (Table 2.10). The bean foliar diseases BCMV had relatively high heritability (85.9 %) while ALS had moderately high heritability of 79.1 %. Diseases CBB and CBR had moderate heritability estimates of 67.6 and 53.3 % respectively (Table 2.10).

Correlations among traits

Overall, significant Pearson correlation coefficients among traits varied from 0.51 to 0.87 for the evaluated traits of the 16 common bean genotypes across the nine locations over two years (Table 2.11). Seed yield was negatively correlated to 100-SW (r= -0.69; *P* value = 0.01) and negatively correlated to both seed K (r= -0.53; *P*= 0.05) and P (r= -0.57; *P*= 0.05) concentrations (Table 2.11). 100-seed weight was negatively correlated to cooking time (r= -0.67; *P*= 0.01), but positively correlated to both seed K (r=0.87; *P* value= 0.001) and P (r=0.66; *P* value= 0.01) concentrations (Table 2.11).

Cooking time was negatively correlated to seed K (r= -0.69; *P* value= 0.01) and P (r= -0.71; *P* value= 0.01). Seed Fe was positively correlated to seed Zn (r=0.62) and Mg (r=0.59) at a significance *P* value of 0.05 for both minerals (Table 2.11). Interestingly, at a *P* value of 0.05 seed Fe was negatively correlated to severity of ALS (r= -0.53) and CBB (r= -0.61). Seed Zn was negatively correlated with CBB (r= -0.51; *P* value = 0.05). Seed K was positively correlated to

seed P (r= 0.83; *P* value=0.001). Seed P was positively correlated with seed Mg (r=0.57; *P* value= 0.05). At a *P* value of 0.01 the foliar disease ALS was positively correlated with CBB (r=0.68) and CBR (r=0.69) (Table 2.11).

Evaluation of genotype and environment performances

Agronomic traits

Yield ranged from 525 - 928 kg ha^{-1} or a 1.8 fold change across genotypes. Highest yield was recorded for genotypes G6 (Chijar), G15 (Amarelo Cela), and G10 (PI527538) as presented in Table 2.12. Genotypes G1 (Uyole 96), G11 (Cebo Cela), and G4 (Charlevoix) had significantly lower yields (Table 2.12). Clearly, genotype G6 (Chijar) from the Carribean and two genotypes G15 (Amarelo Cela) and G10 (PI527538) both landraces from Africa were the highest yielding lines. All the local check varieties (NABE-15, NABE-4, and Masindi yellow) had seed yield lower than the top three genotypes of Chijar, Amarelo Cela, and PI527538 (Tables 2.12 and 2.13).

Differences in environmental mean performance were large for yield with values ranging from 336 to 1057 kg ha^{-1} or a 3.1 fold change. Location KV (Kiyovu in Rakai district) had the highest yield performance followed by the two locations of KA (Kakindo) and TU (Tugonzengane) from Hoima district (Table 2.14). Seed yield was lowest at the two sites TW (Tweweeyo) and KU (Katugezeeku) from Kamuli district. These locations had drier growing seasons (since Kamuli receives less rainfall compared to the other districts in the study) and a high disease pressure for CBB and BCMV.

Genotypic performance for 100-seed weight varied from 25 - 45.3 g. The highest seed weight was observed in genotypes G9 (INIAP425), G1 (Uyole 96), and G5 (Rozi Koko). Lowest 100-SW values were recorded for genotypes G6 (Chijar), G15 (Amarelo Cela), and G7 (Vazon 7).

All the genotypes (G9, G1, G5, and G2) with the highest 100-seed weight mean values were of determinate growth habit while the smallest 100-SW values were recorded in the genotypes G6, G15, and G7 that all belong to the indeterminate growth habit. The 100-seed weight varied from 34.4 to 41.5 g across locations. 100-seed weight was highest for genotypes harvested from KV (Kiyovu) and AG (Agali Awamu) both Rakai district. Genotypes with the smallest 100-SW were from KY (Kyamaleera in Hoima district) and KU (Katugezeeku of Kamuli district).

End use quality

Cooking time varied from 24.6 - 96.5 minutes (3.9 fold variability). The longest cooking genotypes were G15 (Amarelo Cela), G8 (PR0737-1), and G7 (Vazon 7) while the fastest cooking genotypes were G11 (Cebo Cela), G2 (Ervilha), and G9 (INIAP425) (Table 2.12). Two African yellow bean landraces (G2 and 11) along with a white large kidney bean variety G9 (INIAP425) from Ecuador were the fastest cooking lines. The local check varieties had cook times ranging from 35 to 46 minutes (Table 2.13). These shorter cook times make them valuable to the bean growers and consumers. The longest cooking genotype G15 (Amarelo Cela) was also a yellow bean landrace from Africa but also accumulated the highest amount of seed calcium (1889 µg g^{-1}). It will be necessary to further investigate the connection between cooking time and seed calcium concentration in future studies.

Average cooking time variation by location ranged from 38.6 to 65.9 minutes. Genotypes grown at location TW (Tweweeyo in Kamuli district) required the longest time of 65.9 minutes to cook while genotypes grown in KU (Katugezeeku) and (KV) Kiyovu took the shortest time to cook of 38.6 and 39.5 minutes respectively.

Seed nutrient density

Seed Fe varied from 56.6 - 75.4 µg g^{-1} with the genotypes G5 (Rozi Koko), G6 (Chijar), and G11 (Cebo Cela) having the highest Fe concentration. Seed Zn concentration ranged from 26.3 to 34.5 µg g^{-1} with the highest concentration levels found in genotypes G8 (PR0737-1), G5 (Rozi Koko), and G6 (Chijar). In general, red mottled genotypes had more seed Fe and Zn concentration. The local check NABE-15 had a significantly lower amount of seed Fe by ≥ 10 µg g^{-1} and ≥ 3 µg g^{-1} for Zn when compared to the best performers for these two minerals. The other local check NABE-4 and Masindi yellow also had lower concentrations of seed Fe and Zn (Table 2.13). The landrace genotypes G5 (Rozi Koko) and G6 (Chijar) both had higher levels of seed Fe and Zn concentrations presenting the opportunity to biofortify common bean with high amounts of both minerals. Seed Ca concentration ranged from 1099 to 1889 µg g^{-1} with the highest values found in genotypes G15 (Amarelo Cela), G9 (INIAP425), and G3 (Maalasa).

Average seed Fe concentration by location ranged from 58.8 to 72.4 µg g^{-1}. Genotypes grown at KV (Kiyovu) accumulated the largest concentration of seed Fe while genotypes harvested from the TW (Tweweeyo) field location had the lowest seed Fe. It was interesting to note that genotypes G5 (Rozi Koko) grown at KV, G6 (Chijar) grown at GE, and G11 grown at TU had seed Fe concentration ranging from 94 to 103 µg g^{-1} . Across locations, average seed Zn concentration varied from 24.1 to 32.5 µg g^{-1} and genotypes grown at TU (Tugonzengane) had the highest seed Zn. The lowest value in seed Zn concentration was quantified in the seed of genotypes grown at TW (Tweweeyo) in Kamuli district. Genotype G8 (PR0737-1) grown at locations AG and TU had seed Zn concentration ranging from 41 - 43 µg g^{-1} while genotype G5 (Rozi Koko) grown at KY had seed Zn concentration of 43.1 µg g^{-1}. It is interesting to note that, the high yield location (KV) also resulted in seeds with the largest size, fast cooking, and high in seed Fe but low in seed Ca, K, and Mg. This location had a low average soil pH of 5.4, low in soil Ca and K but

high in soil Fe concentrations. The lowest yielding location (TW) resulted in long cooking times, but low in seed Fe, Zn, K, P, and Mg but very high seed Ca concentrations. This location (TW) had a soil pH of 5.8, low soil nitrate, and insufficient soil P and K concentrations while the other soil nutrients were within the optimum range.

Seed Ca concentration ranged from 1098.9 to 1889.1 µg g^{-1} with the highest values found in genotypes G15 (Amarelo Cela), G9 (INIAP425), and G3 (Maalasa). Lowest seed Ca levels were found in genotypes in G14 (Kidungu), G1 (Uyole 96), and G13 (Selian 97). Seed K concentration varied from 2371 to 4107 µg g^{-1} and genotypes with the highest concentration included G9 (INIAP425), G2 (Ervilha), and G5 (Rozi Koko). The lowest seed K concentration was found in genotypes G7 (Vazon 7), G6 (Chijar), and G15 (Amarelo Cela). Seed P ranged from 3565 to 4827 µg g^{-1} and the genotypes with the highest concentration were G9 (INIAP425), G11 (Cebo Cela), and G5 (Rozi Koko). The genotypes with the lowest seed P concentration included G14 (Kidungu), G15 (Amarelo Cela), and G6 (Chijar). Our results showed that some of the high yielding Middle American genotypes (Chijar and Amarelo Cela) also had low levels of seed K and P concentrations. The high yield Middle American yellow had the lowest seed P and K concentrations. Seed Mg concentration varied from 1419 to 1828 µg g^{-1}, with genotypes G11 (Cebo Cela), G5 (Rozi Koko), and G3 (Malaasa) having the highest amount of Mg. The lowest seed Mg concentration was found in landrace G14 (Kidungu), G13 (Selian 97), and variety G12 (Sacramento).

Seed Ca concentration ranged from 1142 to 1479 µg g^{-1} with genotypes grown at location KU (Katugezeeku) having the highest amount of seed Ca across locations. Seed Ca was lowest among common bean genotypes grown at TU (Tugonzengane in Hoima district). Seed K concentration ranged from 2800 to 4210 µg g^{-1} across locations. Common genotypes grown at TU

(Tugonzengane) had the highest amount of seed K while those accessions grown at KA (Kakindo) had the lowest seed K concentration. Seed P concentration varied from 3598 to 4962 µg g^{-1} across locations. The highest mean seed P was observed among genotypes grown at KU (Katugezeeku) while the lowest seed P concentration was recorded in genotypes harvested from TW (Tweweeyo). Seed Mg concentration ranged from 1492 to 1745 µg g^{-1} across locations. The highest value of seed Mg was quantified for field site KU (Katugezeeku) while the lowest amount of seed Mg was recorded for genotypes grown at location TW (Tweweeyo).

Disease scores

For the foliar disease ALS, mean disease ratings varied from 2.7 to 3.7 on a scale of 1 - 9 with genotypes G12 (Sacramento), G2 (Ervilha), and G4 (Charlevoix) showing the highest mean scores. The lowest scores for ALS were found among genotypes G1 (Uyole 96), G6 (Chijar), and G8 (PR0737-1). For CBB, disease ratings varied from 2.5 to 3.4 with genotypes G2 (Ervilha), G12 (Sacramento), and G4 (Charlevoix) having the highest CBB disease scores. The CBB disease scores were smallest within genotypes G5 (Rozi Koko), G6 (Chijar), and G3 (Maalasa). The local check varieties NABE-15 and NABE-4 had high disease scores for ALS and CBB while Masindi yellow had a low score for both diseases in the field (Table 2.13). For CBR, disease ratings ranged from 2.1 to 3.2 with genotypes G4 (Charlevoix), G12 (Sacramento), and G14 (Kidungu) having the highest CBR disease scores. Genotypes G6 (Chijar), G1 (Uyole 96), and G7 (Vazon 7) had the lowest CBR disease ratings (Table 2.12). Both varieties G4 (Charlevoix) and G12 (Sacramento) from the United States had yields <590 kg ha^{-1} and consistently scored highly for ALS, CBB, and CBR foliar diseases in the field (Table 2.12).

The BCMV disease scores ranged from 2.1 to 3.8 on a scale of 1 - 9 and genotypes G11 (Cebo Cela), G7 (Vazon 7), and G15 (Amarelo Cela) had the highest BCMV disease rating values.

Common bean genotypes G6 (Chijar), G14 (Kidungu), and G9 (INIAP425) had the lowest mean scores for BCMV. Field plot mean counts of plants with black root hypersensitive response (BR) ranged from 0 to 5 plants and genotypes G6 (Chijar), G8 (PR0737-1), and G9 (INIAP425) had significantly higher mean plot counts for BR (Table 2.12). Common bean genotypes G2 (Ervilha), G12 (Sacramento), G14 (Kidungu), and G16 (NABE-15 or local check) had clean plots with no plants showing the black root hypersensitive response (Table 2.12). All local check varieties showed no symptoms of black root suggesting absence of the *I* gene in these three varieties (Table 2.13).

Prevalence of ALS foliar disease scores ranged from 2.7 to 4.1 on 1 - 9 disease rating scale. The ALS disease was highly observed among genotypes grown at KY (Kyamaleera) in Hoima district and the lowest ALS scores were recorded among genotypes grown at KU (Katugezeeku) and GE (Tweyunge) field locations both from Kamuli district. The prevalence of CBB disease ranged from 2.7 to 3.3 on a 1 - 9 rating scale. Genotypes grown at locations KA (Kakindo) and TW (Tweweeyo) had the highest levels of CBB while common bean accessions grown at TU (Tugonzengane) had the lowest incidences of CBB disease pressure. Common bean rust disease scores ranged from 2.2 to 3.4 across locations. Highest CBR disease scores were observed in genotype fields grown at location BA (Balitwewunya in Masaka district) while the lowest disease scores were observed at field sites TW (Tweweeyo) and KV (Kiyovu). The prevalence of BCMV disease varied from 2.3 to 3.4 on a scale of 1 - 9. Genotypes grown at the field site BA (Balitwewunya) had the highest scores for BCMV disease while accessions evaluated at KY and TU had the lowest BCMV disease scores (Table 2.14). Genotypes evaluated at location KU (Katugezeeku in Kamuli district) showed the highest mean count of plants with black root

hypersensitive response symptoms. Overall, ALS was the most prevalent foliar disease across sites followed by CBB, CBR and BCMV (Table 2.14).

Polygon ("which-won-where") view of the GGE biplots

The which-won-where GGE biplot is used to detect best performing genotypes in a group of environments or mega-environments for multi-environment field trials (Yan and Tinker, 2005). The polygon is generated by joining extreme genotypes in the study and perpendicular lines (rays) cutting through the sides of the polygon divide groups of genotypes and environments into sectors. When environments fall into different sectors, then different genotypes won in different sectors and a crossover genotype by environment exists (Yan and Tinker, 2006). The wining genotype(s) is/are those located at the vertex of the sector (i.e. the intersection point for the two polygon sides). When all location names fall in one sector then a single genotype was the best performer. Similarly, when genotypes fall in a sector without environments, it implies their performance was below the environmental means and thus performed poorly across all the test environments (Yan and Tinker, 2005).

Yield, cooking time, and seed Fe and Zn concentration

The polygon view of the GGE biplot explained 68, 95, 82, and 82 % of the genotype plus genotype by environment variation for yield, cooking time, seed Fe, and seed Zn concentrations respectively (Figures 2.2 to 2.5). The GGE biplot analysis for yield resulted in two sectors indicating the presence of two winning Middle American genotypes G6 (Chijar), and G15 (Amarelo Cela) for each sector. The presence of two sectors, also confirms presence of genotype x environment interaction and two mega-environments for yield (Figure 2.2). The first mega-environment had locations KY, TU, and KV, while sites BA, AG, GE, TW, KW, KA, and KU

formed the second mega-environments (Figure 2.2). For cooking time, all the environments clustered in one sector (one mega-environment) with one clear slow-cooking genotype G15 (Amarelo Cela) and the fastest cooking genotype was G11 or Cebo Cela (Figure 2.3). For seed Fe concentration all the test environments grouped in one sector and there was one winner G5 (Rozi Koko) (Figure 2.4). For seed Zn, all environments clustered in one sector indicating presence of one mega-environment, and accession G8 (PR0737-1) had significantly higher seed Zn than the other genotypes across the locations (Figure 2.5).

Seed Ca, K, and P concentrations

The which-won-where GGE biplot explained 86, 83, 88, and 86 % of the genotype plus genotype by environment variation for seed Ca, seed K, seed P, and seed Mg concentrations respectively (Figures 2.6 to 2.9). All the environments clustered in one sector for seed Ca concentration implying presence of one mega-environment with one clear winning genotype G15 (Amarelo Cela) (Figure 2.6). This genotype was also the slowest cooking as shown in Figure 2.3 and this suggests there could be some relationship between seed calcium concentration and cooking time.

Genotype mean performance vs. stability GGE biplots

The average environment coordinate (AEC) view (genotype mean performance vs. stability) allows for the comparison of genotypes to be made based on estimates of genotype performance and stability across environments within a mega-environment (Yan et al., 2007). Using this methodology, stability of genotypes is measured by the length of their projection from the AEC horizontal axis (shown by the dotted lines in Figures 2.3 and 2.4). Ideally, genotypes with a near zero projection (i.e. absence of a dotted vertical line above or below the AEC horizontal

axis) are declared stable (Yan et al., 2007). It is suffice to mention that for stability to be meaningful to plant breeders and growers, stable genotypes need to exhibit high trait performances and such results must be confirmed over several years of field evaluations (Yan and Tinker, 2006).

Yield performance

For yield, genotypes towards the direction of G15 (Amarelo Cela) and G6 (Chijar) denote higher yielding accessions whereas genotypes towards the opposite direction represent the poor performing lines G11 (Cebo Cela) and G1 (Uyole 96) (Figure 2.10). Genotypes G3 (Maalasa) and and G14 (Kidungu) were the most stable as they had near zero projection from the AEC horizontal axis. Genotypes G6 (Chijar) and G2 (Ervilha) are regarded as the least stable because they exhibited the longest projection from the AEC horizontal axis. Genotypic performance instability can also indicate local adaptation, whereby genotypes located above or below the AEC horizontal axis would perform better at test environments located in identical orientations of the AEC axis.

Cooking time and seed Fe and Zn concentration

For cooking time, genotypes in the direction of G15 had a higher trait value while genotypes in the opposite direction of the AEC horizontal axis had shorter cooking times with G11 (Cebo Cela) as the fastest cooking line (Figure 2.11). Genotype G8 (PR0737-1) was the most stable for cooking time (near zero projection from the AEC horizontal axis) while genotypes G6 (Chijar) and G15 (Amarelo Cela) were the least stable as they had the longest projections from the AEC horizontal axis (Figure 2.11). Genotype G5 (Rozi Koko) was very stable and also had the highest amount of seed Fe concentration. Genotypes G16 (local check) and G1 (Uyole 96) were the least stable for seed Fe concentration (Figure 2.12). Genotypes G6 (Chijar) and G10 (PI527538) were the most stable for seed Zn concentration as they both had a near zero projection from the AEC

horizontal axis. Genotypes G13 (Selian 97) and G16 (local check) were the least stable (Figure 2.13).

Genotype rankings with reference to the ideal genotype

The ideal genotype is found in the innermost concentric circle where the arrowhead marks the center of that circle. The ideal genotype is supposed to be absolutely stable and high performing for a particular trait of interest to the plant breeder (Yan and Tinker, 2006). Genotypes grouped in the innermost concentric circle have similar performance to the ideal genotype and are therefore the most desirable. Genotypes in the outermost circle are typically less desirable to the plant breeder and can be culled from a breeding program.

Yield and cooking time

In our study, G15 (Amarelo Cela) was closest to the ideal genotype for yield followed by G6 (Chijar) making them the most desirable accessions based on the GGEBiplot analysis while genotypes G1 (Uyole 96) and G11 (Cebo Cela) were the furthest from the ideal genotype and are thus the most undesirable (Figure 2.18). For cooking time, none of genotypes made it into the innermost concentric circle with the ideal genotype, but genotypes G15 (Amarelo Cela) and G8 (PR0737-1) were in the second concentric circle when the ideal genotype was considered to be the longest cooking bean accession (Figure 2.19). But it was also clear that the fastest cooking beans G11 (Cebo Cela) and G2 (Ervilha) were in the outermost (6^{th}) circle and these would be genotypes desirable to the bean growers and consumers since fast cooking is a highly desirable consumer trait in common bean (Figure 2.19).

Seed Fe and Zn concentration

For seed Fe concentration, two genotypes G5 (Rozi Koko) and G11 (Cebo Cela) fell inside the circle with the ideal genotype with accession G6 (Chijar) right on the edge of the first circle. Therefore, all these three genotypes are high in seed Fe. The genotype with the lowest seed Fe was G14 (Kidungu) (Figure 2.20). Genotypes G8 (PR0737-1) followed by G5 (Rozi Koko) and G6 (Chijar) were highest seed Zn in our study (Figure 2.20). Genotype G14 (Kidungu) was the least desirable for both seed Fe and Zn concentrations (Figures 2.20 and 2.21).

Discussion

From the ANOVA table for yield, both the location and the location x season components were important sources of variation. Yield in common bean is a highly complex trait and affected by environmental factors (Kelly et al., 1998; Checa and Blair, 2012) as shown by our results. This suggests that dry bean breeders should either develop varieties adapted to specific locations or stable varieties that can perform well over wide ranging agro-ecological conditions. There were also large differences in mean performance for yield across locations, which further reinforce the need to develop location-adapted common bean cultivars for Uganda.

The ANOVA for 100-seed weight showed that most of the variability was due to the genetics (51.5 %). In common bean seed size is strongly tied to gene pool structure where Andean beans are usually larger compared to the small-seeded genotypes of the Middle America genotypes (Singh et al., 2002). The 16 genotypes in our study were comprised of 11 determinate and five indeterminate, where most of the determinate were large kidney seed types and majority of them weighed ≥ 35 g per 100 seeds which is typical of large seeded Andean beans (Beaver and Kelly, 1994).

Most of the variability in cooking time was due to genotypes indicating that the genotypes were diverse with large differences among means for this trait. This implies it is plausible to

develop common bean varieties that can cook faster for Uganda based on the variability uncovered in the study genotypes.

Both seed Fe and Zn concentration were largely affected by the genotype and location effects. Soil chemical composition properties and weather patterns have been known to influence nutrient uptake in plants as these affect nutrient solubility and availability in the root zone (Manzeke et al., 2012). In our study, one of the sites KV that resulted in high seed Fe, (Table 2.14) also had the highest mean soil Fe in both years. A similar pattern was observed for seed Zn where location KY with high mean seed Zn (Table 2.12) also had high soil Zn at planting in 2016 (Table 2.4).

The very strong influence of genotype and location components on seed P concentration suggests that breeding effects for this trait should focus on developing location-adapted varieties. Indeed, low phosphorus is a big challenge on agricultural lands in Africa where low/no use of phosphorus based fertilizers is very common (Nziguheba, 2007). Identification of common bean genotypes that can perform well under low soil P conditions should be a desired goal for sub-Saharan Africa.

The common bean foliar diseases (ALS and CBR) were controlled mostly by effects of location and genotype x location. This implies there were differences in disease pressures and weather patterns that could have all influenced the genotypes' response to field infestations. Since infections were not in a controlled environment and were reliant on pathogen races present in the trial areas, there could have been different or multiple races present at the nine locations. Locational differences in weather (wet vs. dry) can lead to accelerated / restricted sporulation thus influencing disease buildups (Olango et al., 2017; Pyndji and Trutmann, 1992).

The impact of the genotype x location was the most substantial component for CBB disease followed by genotype x location x season and then genotype x location. This indicates disease varied across locations and could be due to variation in weather patterns across our trial location that influenced development and spread of the bacterial pathogen throughout the research plots. Presence of unknown resistance genes in our accessions might have resulted in the differences observed with regards to genotype reactions to CBB pressure (Hailu et al., 2017). Most of the observed differences in BCMV mean scores is attributable to genotype, as genotypic difference was the major source of variation. Location and genotype x location also controlled the mean scores for BCMV among the 16 genotypes.

Black root (BR) condition is a hypersensitive response that happens in common bean varieties carrying the *I* gene for protection against strains of BCMV disease (Ali, 1950). When plants carrying the unprotected *I* gene are challenged with strains of the bean common mosaic necrosis virus (BCMNV), it triggers systemic vascular necrosis that causes wilting and plant death (Kelly et al., 1995). The BCMNV disease is endemic to regions of eastern and central Africa (Sibernagel et al., 1986). Since the *I* gene has been widely deployed in germplasm developed in North America, we were interested to know which genotypes would show the black root symptoms. Genotypes G6 (Chijar), G8 (PR0737-1), and G9 (INIAP425) had the highest mean number of counts with BR hypersensitive response (Table 2.12). Both genotypes G6 and G8 are from the Caribbean (Table 2.1) and suffered the most from strains of BCMNV infection in the field. If these genotypes are to be further utilized in East Africa, the *I* gene will be need to be protected with the *bc-3* locus so that they can maintain their productivity potential especially since G6 (Chijar) was highly productive across all the nine locations.

The local check bean genotypes had below average performances for yield and accumulated low levels of seed Fe and Zn concentrations compared to most of our test genotypes. However, they were fast cooking which is one of the reasons the growers in Uganda like them. They also had high mean disease incidences especially for ALS and CBB. Since farmers value high yielding, fast cooking and nutrient dense beans, the germplasm identified in this study can be used to make informed crossing blocks for developing bean varieties that can meet the needs of the Ugandan smallholder farmers. For Uganda, focus should be on yield followed by careful introgression of alleles controlling Fe, Zn, and cooking time into the desired seed type preferably yellows and red mottled. Our study showed presence of two mega environments for yield and one mega-environment for cooking time, and seed Fe and Zn concentrations. Mega-environment identification has vital implications with regards to selection of test locations, genotype evaluation and selection, and variety release in a breeding program. Based on results from this study it appears that evaluation of genotypes for yield should be conducted in two mega-environments like locations KY or KV would give similar variety performance information. For the second mega-environment for yield, locations GE or BA would give similar variety performance information. With one mega-environment present for cooking time, seed Fe and Zn concentrations, locations KY and TU would give representative information on genotype performance for these traits since they are centrally located in the single sector (mega-environment) of the "which won where" GGE biplot (Figures 2.3 to 2.5). Evaluating genotypes in fewer locations would maximize resources and speed-up genotype selections and variety release.

From a plant breeding perspective, correlation analysis can help identify positive or negative relations among traits, identify novel parental combinations for variety development, and detect trait measurement redundancy (Yan and Fregeau-Reid, 2008). Our results showed that yield

was positively correlated to cooking time but negatively correlated to 100-seed weight, seed K, and seed P concentrations. Two of the small seeded Middle American landraces (Chijar and Amarelo Cela) were the highest yielding in our nine-location trial. Previous studies have also showed that increasing seed size results in a yield penalty and this has been one of the major obstacles with regards to yield improvements in the Andean common bean gene pool lagging behind those of cultivars from the Middle American gene pool (Singh et al., 2002). We also showed that seed Fe was positively correlated with seed Zn concentration based on field trial data from Uganda. This is particularly exciting since a number of dry bean biofortification efforts would like to develop varieties that are high in both Fe and Zn concentrations. We actually identified two landrace genotypes G5 (Rozi Koko) and G6 (Chijar) which had the highest concentrations of both seed Fe and Zn (Table 2.12). This high positive correlation among seed Fe and Zn suggests that these two phenotypes might have evolved together and are potentially under similar genetic control of ion transporters and transcription factors related to mineral movement in the bean plants (Baxter, 2015).

Conclusion

We identified high yielding genotypes from our study where the landrace G6 (Chijar) was consistently very productive across the nine locations in Uganda. Two genotypes G5 (Rozi Koko) and G6 (Chijar) had a high combination of both seed Fe and Zn concentrations. These could be promising accessions for mineral biofortification breeding work. Fast cooking lines were African landraces G2 (Ervilha) and G11 (Cebo Cela), and the Ecuadorian white kidney variety G9 (INIAP425). These lines will be useful in future common bean breeding efforts to develop varieties that require less time to cook. The high broad-sense heritability estimates indicate that genetic variability for most traits exists within the study genotypes. Therefore, these genetic materials will

be useful as choice parents for improving dry beans for the evaluated traits. Presence of mega-environments for traits like yield, cooking time, seed Fe and Zn concentrations in Uganda, implies that common bean breeders will need to develop bean varieties adapted to particular mega-environments or cultivars that are high performing and stable across a wide-range of agro-ecological conditions.

Table 2.1: Description of the 15 genotypes evaluated in the study in Uganda

ID code	ADP code	Name	Gene pool	Region of origin	Origin	Cultivation status	Seed type	Seed size (Avg. 100 seed wt) (g)	Growth habit
G1	ADP0112	Uyole 96	Andean	East Africa	Tanzania	Variety	DRK	44.7	Determinate
G2	ADP0512	Ervilha	Andean	Southern Africa	Angola	Landrace	Yellow	42.2	Determinate
G3	ADP0009	Maalasa	Andean	East Africa	Tanzania	Landrace	Red mottled	40.1	Determinate
G4	ADP0598	Charlevoix	Andean	North America	US	Variety	DRK	41.6	Determinate
G5	ADP0001	Rozi Koko	Andean	East Africa	Tanzania	Landrace	Red mottled	42.4	Determinate
G6	ADP0445	Chijar	Middle American	Caribbean	Puerto Rico	Landrace	Red mottled	25	Indeterminate
G7	ADP0443	Vazon 7	Middle American	Caribbean	Puerto Rico	Landrace	Red mottled	30.4	Indeterminate
G8	ADP0434	PR0737-1	Andean/Admix	Caribbean	Puerto Rico	Variety	Red mottled	34.2	Indeterminate
G9	ADP0452	INIAP425	Andean	South America	Ecuador	Variety	White	45.3	Determinate
G10	ADP0468	PI527538	Andean	East Africa	Burundi	Landrace	Yellow	40.1	Determinate
G11	ADP0521	Cebo Cela	Andean	Southern Africa	Angola	Landrace	Yellow	35.2	Indeterminate
G12	ADP0602	Sacramento	Andean	North America	US	Variety	LRK	40.3	Determinate
G13	ADP0098	Selian 97	Andean	East Africa	Tanzania	Variety	DRK	35.2	Determinate
G14	ADP0003	Kidungu	Andean	East Africa	Tanzania	Landrace	Small red	34.1	Determinate
G15	ADP0522	Amarelo Cela	Middle American	Southern Africa	Angola	Landrace	Yellow	26.6	Indeterminate

DRK: Dark red kidney; *LRK:* Light red kidney

Table 2.2: Description of the local checks evaluated along with the ADP test genotypes in the study in Uganda

Genotype code	Name	Locations planted	Gene pool	Region of origin	Origin	Cultivation status	Seed type	Seed size (Avg. 100 seed wt) (g)	Growth habit
NABE-15	Kanyeebwa	KA, TU, KU, GE, KV, and BA	Andean	East Africa	Uganda	Variety	Cream mottled	40.5	Determinate
-	Masindi yellow	KY and TW	Andean	East Africa	Uganda	Landrace	Yellow	37.6	Determinate
NABE-4	Red mottled	AG	Andean	East Africa	Uganda	Variety	Red mottled	44.7	Determinate

Table 2.3: Description of the nine locations used for the genotype x environment study in Uganda

District	Annual rainfall range (mm)	Annual temperature range (°C)	Agro-ecological zone	Location	Location code	Geographic coordinate		Altitude (m asl)	Soil type
Hoima	800 - 1,400	15 - 30	Grass land savanna	Kakindo	KA	N01°28.54′	E031°25.46′	1,228	Sandy clay
				Kyamaleera	KY	N01°29.47′	E031°26.99′	1,174	Sandy clay loam
				Tugonzengane	TU	N01°16.93′	E031°17.77′	1,138	Clay
Kamuli	800 - 1,300	16 - 31	Tall savanna	Katugezeeku	KU	N00°50.60′	E033°12.11′	1,127	Clay loam
				Tweweeyo	TW	N00°54.79′	E033°01.33′	1,086	Sandy clay loam
				Tweyunge	GE	N00°53.77′	E032°59.94′	1,061	Sandy clay loam
Rakai	850 - 2,125	15 - 27	Tropical rain forest	Agali Awamu	AG	S00°34.87′	E031°34.19′	1,233	Sandy clay
				Kiyovu	KV	S00°43.58′	E031°29.27′	1,215	Sandy clay loam
Masaka	850 - 2,125	15 - 27	Tropical rain forest	Balitwewunya	BA	S00°25.54′	E031°38.14′	1,249	Clay

Table 2.4: Soil chemical composition analysis for the nine study locations over the two years in Uganda

District	Location	Year	Soil nutrient levels at planting								
			pH	OM (%)	Nitrate (µg g^{-1})	P (µg g^{-1})	Mg (µg g^{-1})	K (µg g^{-1})	Ca (µg g^{-1})	Zn (µg g^{-1})	Fe (µg g^{-1})
Hoima	KA	2015	6	6.7	21.2	12	453	144	1,998	1.9	7.1
		2016	5.7	4.3	5.4	6	223	76	1,135	1.4	11
	KY	2015	5.9	5.6	37.6	8	303	216	1,958	1.3	8.3
		2016	6.3	3	4.4	36	170	45	1,482	4.4	8.5
	TU	2015	6.2	3.7	22.9	10	183	195	1,283	2.1	8.2
		2016	5.5	6.5	14.9	20	281	212	1,107	2.1	17
Kamuli	KU	2015	6.1	3	50.3	12	188	239	1,377	2.6	9.2
		2016	5.7	4.6	16.3	13	237	83	1,158	1.9	21
	TW	2015	6	4.8	8.3	13	278	94	1,509	1	9
		2016	5.6	2.8	8.9	7	129	68	705	4.3	17
	GE	2015	6	3.6	14.6	20	239	531	1,376	3.3	12
		2016	7.1	3.5	11.9	23	222	305	2,038	3.1	7.8
Rakai	AG	2015	5	2.9	25.7	14	98	28	444	0.9	23
		2016	5.5	4.3	11.8	23	209	83	1,019	2.8	22
	KV	2015	5.4	4.2	13.5	20	187	63	951	0.9	29
		2016	5.5	2.9	6.3	21	95	172	833	4.2	18
Masaka	BA	2015	5.2	4.6	22.9	36	298	171	1,290	3	22
		2016	5.6	5.1	16.4	13	268	124	1,379	1.7	11

Table 2. 5: ANOVA showing mean squares and percentage of total variance explained for yield, 100-seed weight, and cooking time of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda

		Traits					
		Yield (kg ha^{-1})		100-seed weight (g)		Cooking time (min)	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained
G	15	558104.2***	4.8	1351.4***	51.5	12850.9***	40.6
L	8	4738947.6***	21.5	327.5***	6.7	4502.0***	7.6
S	1	719139.3***	0.4	515.1***	1.3	16324.3***	3.4
GxL	120	100884.9***	6.9	16.0**	4.9	763.0***	19.3
GxS	15	169218.6***	1.4	48.0***	1.8	3606.8***	11.4
LxS	8	10706389.0***	48.6	981.6***	19.9	665.5***	1.1
rep(LxS)	18	146755.7***	1.5	16.2 NS	0.7	55.3NS	0.2
GxLxS	120	100468.7***	6.8	18.6***	5.7	549.6***	13.9

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 2.6: ANOVA showing mean squares and percentage of total variance explained for seed iron, zinc, and calcium concentrations of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda

		Traits					
		Fe (µg g^{-1})		Zn (µg g^{-1})		Ca (µg g^{-1})	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean Square	Percentage of TSS explained	Mean square	Percentage of TSS explained
G	15	1064.3***	27.0	172.8***	23.1	1674481.3***	41.8
L	8	1111.3***	15.0	357.4***	25.5	822039.9***	11.0
S	1	5968.9***	10.1	157.9***	1.4	90799.3NS	0.2
GxL	120	56.6***	11.5	9.6***	10.3	60503.0***	12.1
GxS	15	85.8***	2.2	14.0***	1.9	281344.3***	7.0
LxS	8	773.2***	10.5	231.2***	16.5	106706.2***	1.4
rep(LxS)	18	15.3NS	0.5	7.3NS	1.2	39378.4NS	1.2
GxLxS	120	66.5***	13.5	6.9*	7.4	61180.1***	12.2

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 2.7: ANOVA showing mean squares and percentage of total variance explained for seed potassium, phosphorus, and magnesium concentrations of 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda

		Traits					
		K (µg g^{-1})		P (µg g^{-1})		Mg (µg g^{-1})	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained
G	15	8769285.8***	23.2	5200376.2***	30.7	484471.5***	32.6
L	8	11563264.1***	16.3	10553615.3***	33.3	382200.2***	13.7
S	1	4540805.8***	0.8	43649.7NS	0.0	921344.0***	4.1
GxL	120	875386.5***	18.5	233392.9***	11.0	19202.1***	10.3
GxS	15	1153767.2***	3.1	511894.9***	3.0	89550.5***	6.0
LxS	8	3469298.7***	4.9	1108590.2***	3.5	364907.8***	13.1
rep(LxS)	18	317589.8NS	1.0	160891.5*	1.1	15312.7**	1.2
GxLxS	120	618365.0**	13.1	173228.6***	8.2	19182.2***	10.3

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 2.8: ANOVA showing mean squares and percentage of total variance explained for the bean foliar diseases angular leaf spot, common bacterial blight, and common bean rust among 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda

		Traits					
		ALS (1-9)		CBB (1-9)		CBR (1-9)	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained
G	15	3.2***	9.1	2.0***	8.7	2.6***	8.7
L	8	16.0***	24.3	3.3***	7.6	11.6***	21.0
S	1	38.5***	7.3	13.1***	3.8	27.1***	6.1
GxL	120	0.7***	15.3	0.5**	17.1	0.8***	21.1
GxS	15	0.4NS	1.2	0.6**	2.8	0.8***	2.7
LxS	8	8.1***	12.3	6.8***	15.7	6.3***	11.3
rep(LxS)	18	0.9***	3.0	0.6*	2.9	0.5*	1.9
GxLxS	120	0.5***	11.9	0.5**	16.6	0.4**	10.4

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 2.9: ANOVA showing mean squares and percentage of total variance explained for the bean common mosaic virus and black root among 16 common bean genotypes evaluated for two field seasons at nine locations in Uganda

		Traits			
		BCMV (1-9)		BR (plant count)	
Source of variation	DF	Mean square	Percentage of TSS explained	Mean square	Percentage of TSS explained
G	15	5.0***	22.0	49.9***	16.5
L	8	7.3***	17.2	53.3***	9.4
S	1	0.7NS	0.2	2.4NS	0.1
GxL	120	0.5***	16.0	17.8***	47.0
GxS	15	0.6**	2.7	3.2*	1.0
LxS	8	2.6***	6.0	21.9***	3.9
rep(LxS)	18	0.3NS	1.6	1.0NS	0.4
GxLxS	120	0.4*	12.5	4.5***	11.8

Significance level: * = *P* value <0.05; ** = *P* value <0.01; *** = *P* value <0.001; NS = not significant

Table 2.10: Descriptive summary statistics and broad sense heritability estimates for agronomic, cooking time, nutrient composition traits, and response to biotic stresses of 16 common bean genotypes grown across nine locations for two years in Uganda

Traits	Mean (± SD)	Min.	Max.	Fold variation	Broad sense heritability (H^2) (%)
Yield (kg ha^{-1})	660 ± 125	525	929	1.8	69.7
100-seed weight (g)	37.4 ± 6.1	25	45.3	1.8	96.5
Cook time (mins)	45.6 ± 18.9	24.6	96.5	3.9	70.3
Fe (µg g^{-1})	65.2 ± 5.4	56.6	75.4	1.3	91.9
Zn (µg g^{-1})	29.4 ± 2.2	26.3	34.5	1.3	90.3
Ca (µg g^{-1})	1348 ± 216	1099	1889	1.7	83.9
K (µg g^{-1})	3214 ± 494	2371	4107	1.7	83.9
P (µg g^{-1})	4124 ± 380	3565	4827	1.4	89.2
Mg (µg g^{-1})	1595 ± 116	1419	1828	1.3	81.5
ALS (1-9)	3.2 ± 0.3	2.7	3.7	1.4	79.1
CBB (1-9)	2.9 ± 0.3	2.5	3.5	1.4	67.6
CBR (1-9)	2.6 ± 0.3	2.1	3.2	1.5	53.3
BCMV (1-9)	2.7 ± 0.4	2.1	3.8	1.8	85.9
BR (plant count)	1.3 ± 1.4	0	5	-	-

ALS: Angular leaf spot; *CBB*: Common bacterial blight; *CBR*: Common bean rust; *BR*: Black root hypersensitive response; *SD*: standard deviation of the mean; *Min* and *Max* refers to ranges for each trait; *Fold variation* refers to maximum value relative to the minimum value for every trait

Table 2.11: Pairwise correlation coefficients among traits averaged over the nine locations and two field seasons

Traits	100-SW	Cook time	Fe	Zn	Ca	K	P	Mg	ALS	CBB	CBR	BCMV
Yield	-0.69**	0.58*	0.04 ns	0.08 ns	0.44 ns	-0.53*	-0.57*	-0.09 ns	-0.09 ns	-0.33 ns	-0.46 ns	-0.33 ns
100-SW	-	-0.67**	0.01 ns	-0.21 ns	-0.19 ns	0.87***	0.66**	0.17 ns	0.27 ns	0.38 ns	0.39 ns	-0.09 ns
Cook time		-	-0.19 ns	0.20 ns	0.40 ns	-0.69**	-0.71**	-0.28 ns	-0.33 ns	-0.06 ns	-0.12 ns	0.08 ns
Fe			-	0.62*	-0.01 ns	0.26 ns	0.41 ns	0.59*	-0.53*	-0.61*	-0.30 ns	0.1 ns
Zn				-	-0.20 ns	-0.22 ns	0.01 ns	0.10 ns	-0.47 ns	-0.51*	-0.29 ns	0.11 ns
Ca					-	0.11 ns	0.07 ns	0.25 ns	-0.13 ns	0.01 ns	-0.09 ns	-0.03 ns
K						-	0.83***	0.47 ns	0.19 ns	0.22 ns	0.25 ns	-0.05 ns
P							-	0.57*	0.30 ns	0.12 ns	0.23 ns	0.24 ns
Mg								-	-0.30 ns	-0.34 ns	-0.11 ns	0.36 ns
ALS									-	0.68**	0.69**	0.13 ns
CBB										-	0.45 ns	0.20 ns
CBR											-	0.04 ns
BCMV												-

* Significance at the 0.05 probability level. ** Significance at the 0.01 probability level. *** Significance at the 0.001 probability level. ns, not significant

Table 2.12: Genotype means for the observed traits of 16 common bean genotypes evaluated across nine field sites for two years in Uganda

Genotype	Yield (kg ha^{-1})	Seed weight (g)	Cook time (mins)	Fe (µg g^{-1})	Zn (µg g^{-1})	Ca (µg g^{-1})	K (µg g^{-1})	P (µg g^{-1})	Mg (µg g^{-1})	ALS (1-9)	CBB (1-9)	CBR (1-9)	BCMV (1-9)	BR (plant count)
G1	525	44.7	41.3	69.6	30.2	1134	3531	4096	1567	2.7	3.1	2.3	2.9	1
G2	671	42.2	27.0	61.4	27.3	1518	3771	4370	1599	3.5	3.4	2.7	2.7	0
G3	628	40.1	35.8	63.0	27.4	1559	3550	4168	1708	3.0	2.7	2.6	2.6	1
G4	536	41.6	48.9	64.4	29.1	1234	3538	4249	1592	3.5	3.2	3.2	2.7	1
G5	589	42.4	32.8	75.4	32.4	1256	3638	4518	1762	2.9	2.5	2.8	2.7	1
G6	929	25.0	53.8	72.8	31.9	1366	2564	3781	1611	2.7	2.5	2.1	2.1	5
G7	645	30.4	65.0	59.0	30.7	1272	2371	3831	1594	3.1	3.0	2.4	3.4	1
G8	709	34.2	72.4	67.4	34.5	1418	2871	3850	1516	2.8	2.8	2.5	2.8	3
G9	593	45.3	28.2	66.6	27.7	1672	4107	4827	1684	3.1	3.0	2.5	2.5	2
G10	786	40.1	43.9	62.2	27.6	1184	3215	3867	1665	3.0	3.0	2.6	2.6	1
G11	531	35.2	24.6	73.7	29.9	1261	3461	4767	1828	3.3	2.9	2.6	3.8	1
G12	587	40.3	43.1	60.6	29.0	1308	3086	4319	1450	3.7	3.3	3.1	2.7	0
G13	714	35.2	33.1	65.4	29.4	1147	2937	3943	1423	3.3	2.8	2.5	2.6	1
G14	561	34.1	44.8	56.6	26.3	1099	2688	3565	1419	3.3	3.0	2.8	2.4	0
G15	910	26.6	96.5	63.5	27.4	1889	2654	3590	1586	3.0	3.0	2.6	3.0	1
G16	735	40.5	35.7	62.5	29.1	1257	3551	4439	1547	3.5	3.1	2.6	2.8	0
LSD (α=0.05)	**107**	**1.5**	**3.0**	**2.2**	**1.1**	**79**	**293**	**136**	**39**	**0.3**	**0.3**	**0.2**	**0.2**	**0.6**

LSD: Least significance difference used to compare genotype performances for the 14 measured traits; G16 denotes the NABE-15 local check variety that was grown at locations KA, TU, KU, GE, KV, and BA.

Table 2.13: Mean performance of the local check common bean varieties for the observed traits evaluated for two years in Uganda

Local check	Yield (kg ha^{-1})	Seed weight (g)	Cook time (mins)	Fe (µg g^{-1})	Zn (µg g^{-1})	Ca (µg g^{-1})	K (µg g^{-1})	P (µg g^{-1})	Mg (µg g^{-1})	ALS (1-9)	CBB (1-9)	CBR (1-9)	BCMV (1-9)	BR (plant count)
NABE-15	735	40.5	35.7	62.5	29.1	1257	3551	4439	1547	3.5	3.1	2.6	2.8	0
NABE-4	472	44.7	38.2	65.2	32.9	1283	3399	4013	1476	3.6	3.3	2.0	2.4	0
Masindi yellow	439	37.6	45.6	54.0	25.7	1207	3125	3745	1483	2.8	3.0	2.5	2.8	0

NABE-15 local check was grown at locations KA, TU, KU, GE, KV, and BA; NABE-4 was evaluated at location AG while Masindi yellow was grown at locations KY and TW

Table 2.14: Environmental means for the measured traits across 16 common bean genotypes evaluated in nine field sites for two years in Uganda

Location	Yield (kg ha^{-1})	Seed weight (g)	Cook time (mins)	Fe (µg g^{-1})	Zn (µg g^{-1})	Ca (µg g^{-1})	K (µg g^{-1})	P (µg g^{-1})	Mg (µg g^{-1})	ALS (1-9)	CBB (1-9)	CBR (1-9)	BCMV (1-9)	BR (plant count)
KA	897	37.6	43.0	63.7	30.1	1235	2800	3998	1598	3.5	3.3	2.4	2.6	1
KY	695	34.4	50.6	65.5	30.5	1370	3363	3913	1632	4.1	3.1	3.2	2.3	1
TU	981	35.4	44.2	66.1	32.5	1142	4210	4447	1660	2.9	2.7	2.8	2.4	1
KU	350	34.8	38.6	68.4	31.0	1479	3148	4962	1745	2.7	2.8	2.5	3.1	3
TW	336	37.1	65.9	58.8	24.1	1439	2818	3598	1492	2.9	3.3	2.2	2.9	1
GE	666	38.4	42.4	67.3	28.4	1417	3304	4094	1566	2.7	2.8	2.3	2.8	1
AG	418	39.2	44.9	59.8	29.7	1388	3268	3743	1536	2.8	2.8	2.6	2.5	1
KV	1057	41.5	39.5	72.4	28.9	1244	2980	4070	1527	3.7	3.0	2.2	2.8	1
BA	637	37.9	41.1	64.4	29.9	1417	3034	4289	1603	3.2	2.9	3.4	3.4	1
LSD (α=0.05)	**80**	**1.2**	**2.3**	**1.6**	**0.8**	**59**	**220**	**102**	**29**	**0.2**	**0.2**	**0.2**	**0.2**	**0.5**

LSD: Least significance difference used to compare environmental performances for the 14 measured traits

Figure 2.1: Map of Uganda showing the study districts of Hoima, Kamuli, Masaka, and Rakai for the multi-location on-farm evaluation of the 16 common bean genotypes for two field seasons of 2015 and 2016

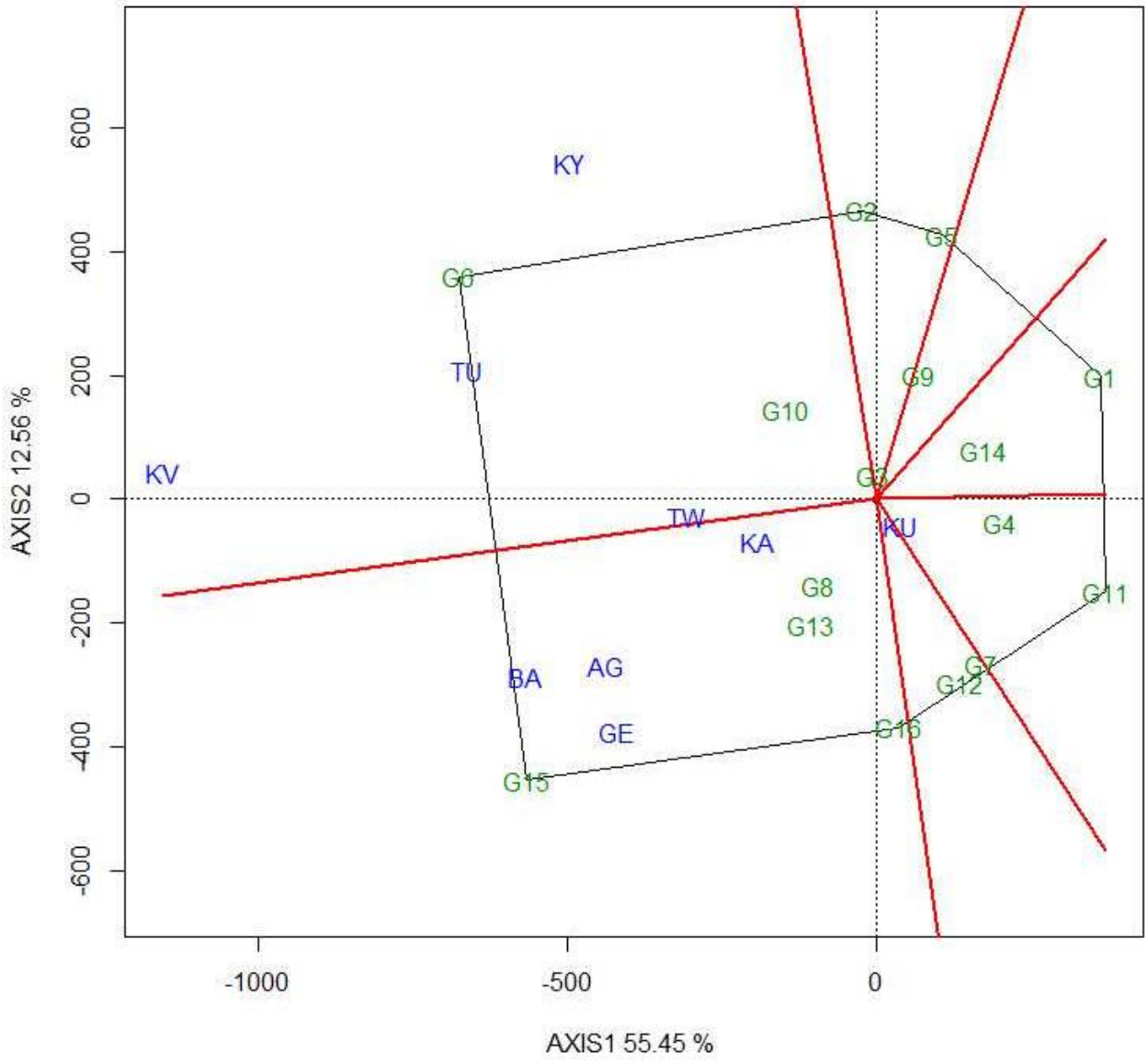


Figure 2.2: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

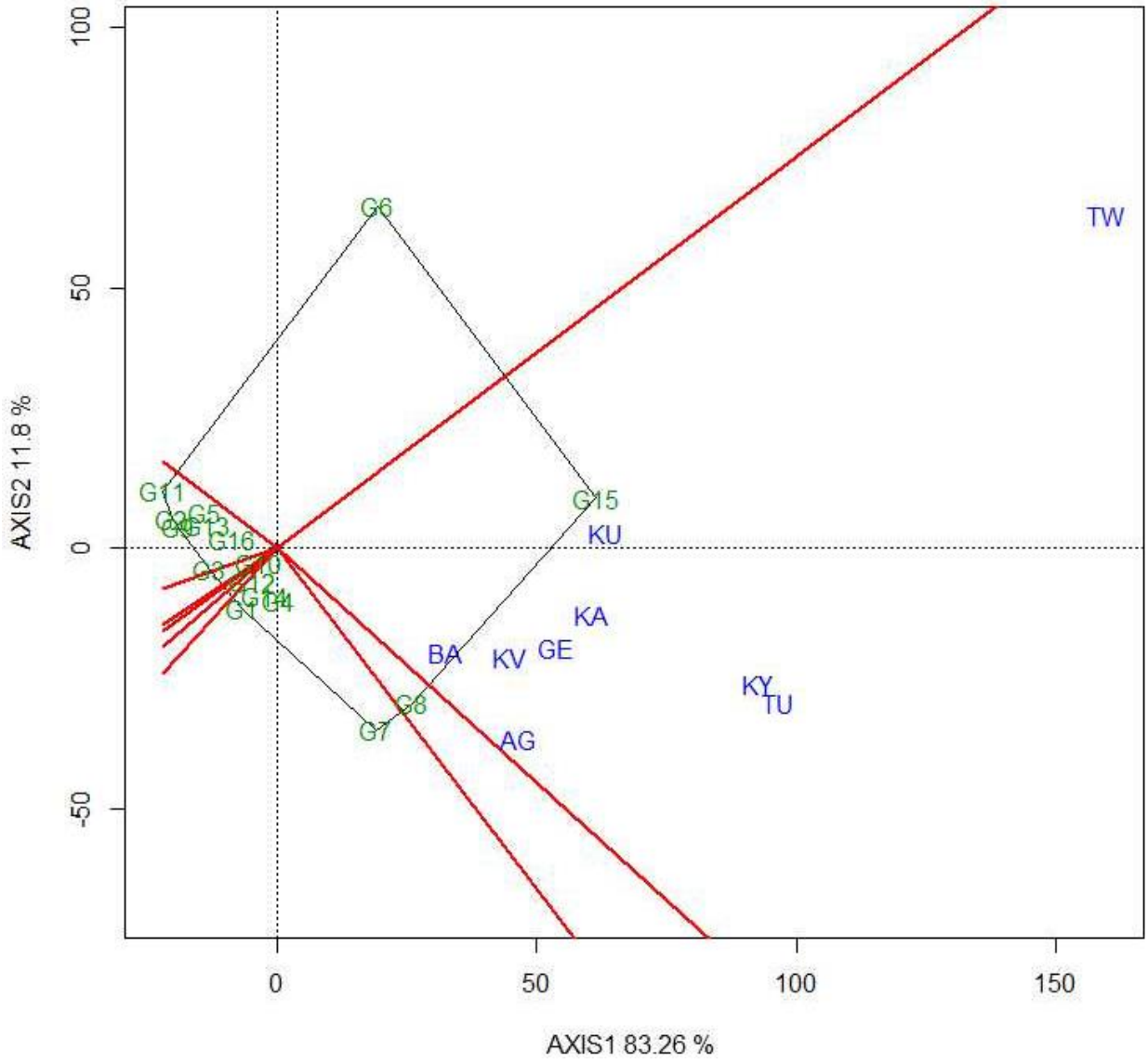


Figure 2.3: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively**.**

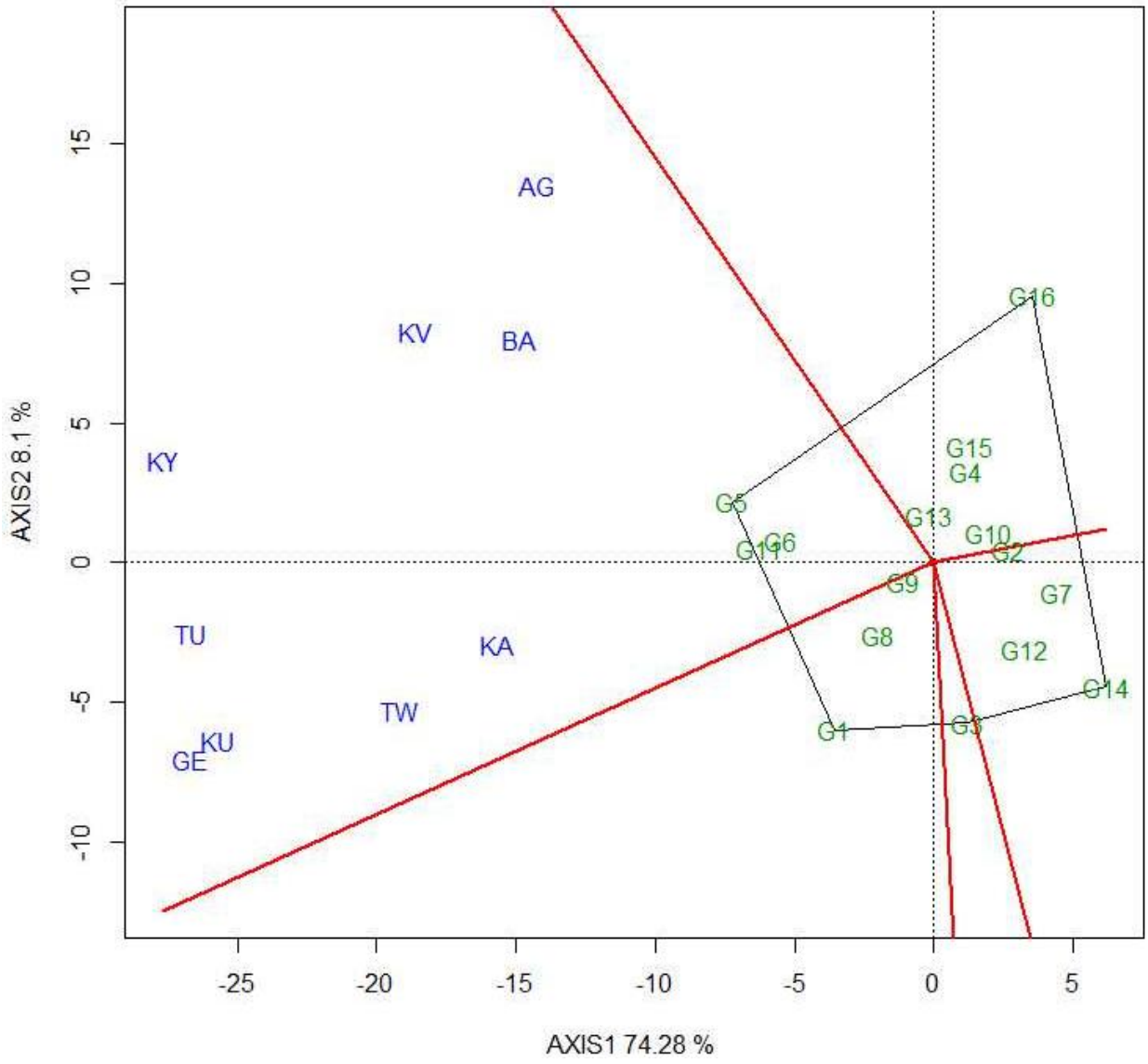


Figure 2.4: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

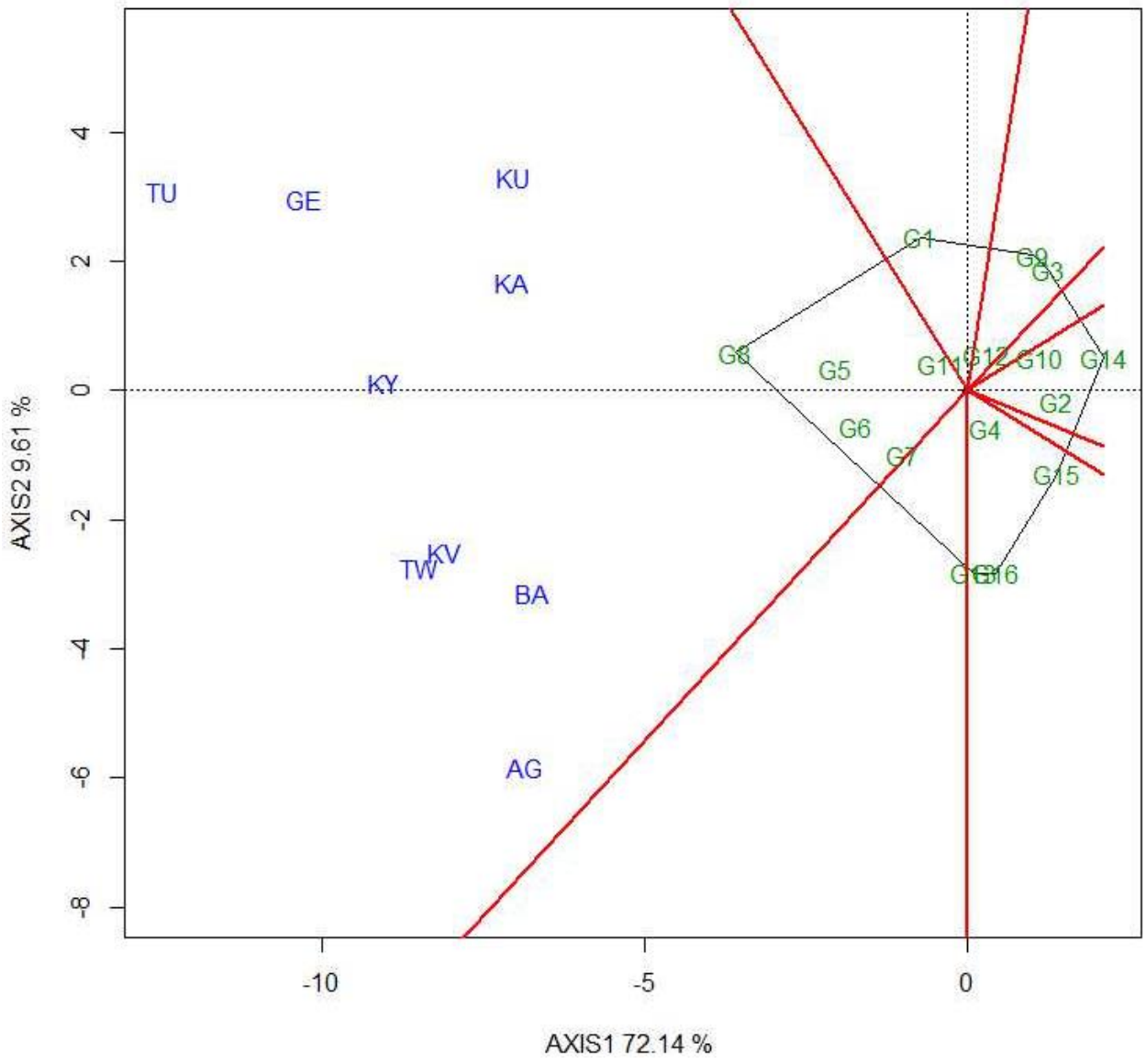


Figure 2.5: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

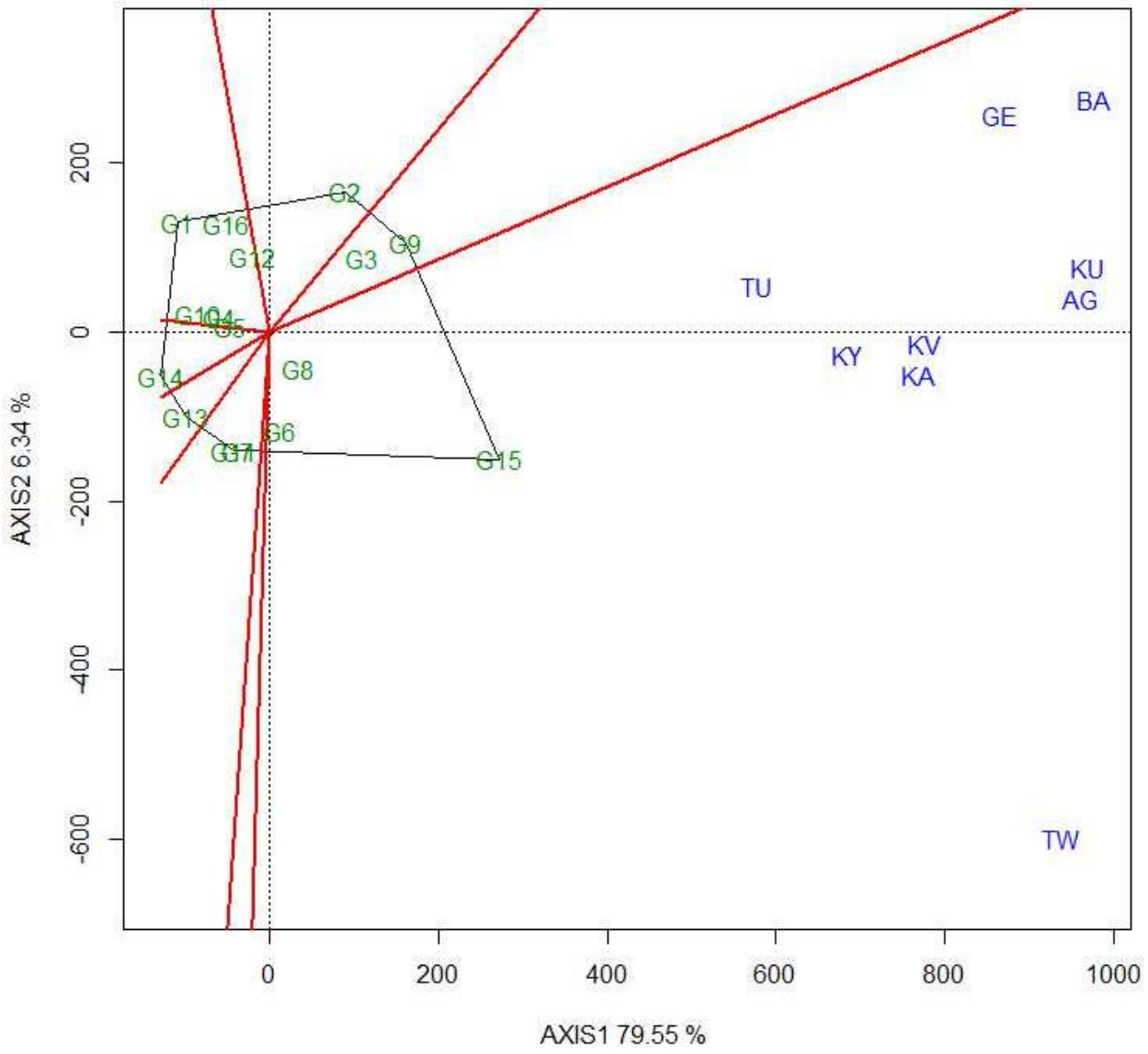


Figure 2.6: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

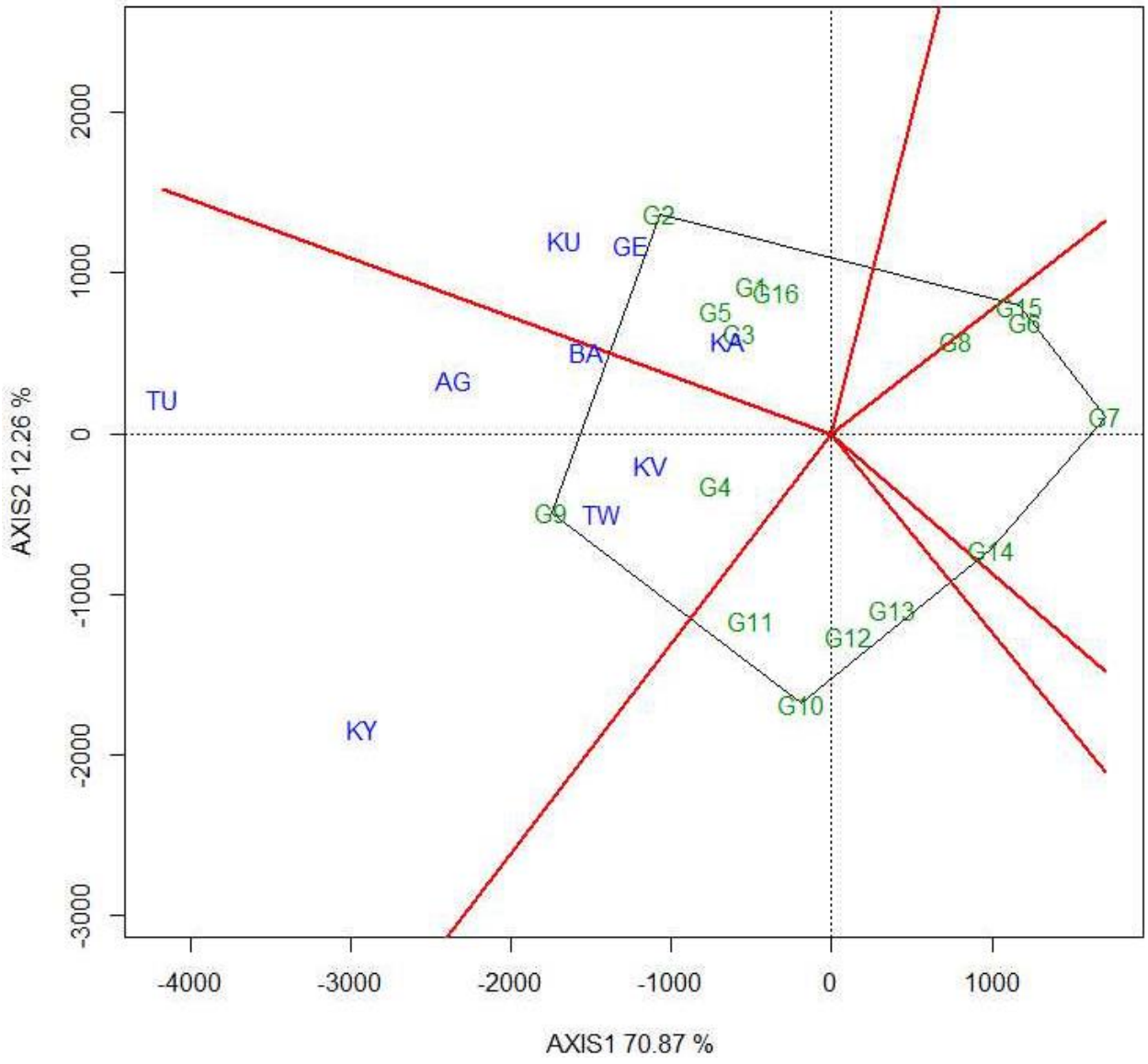


Figure 2.7: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

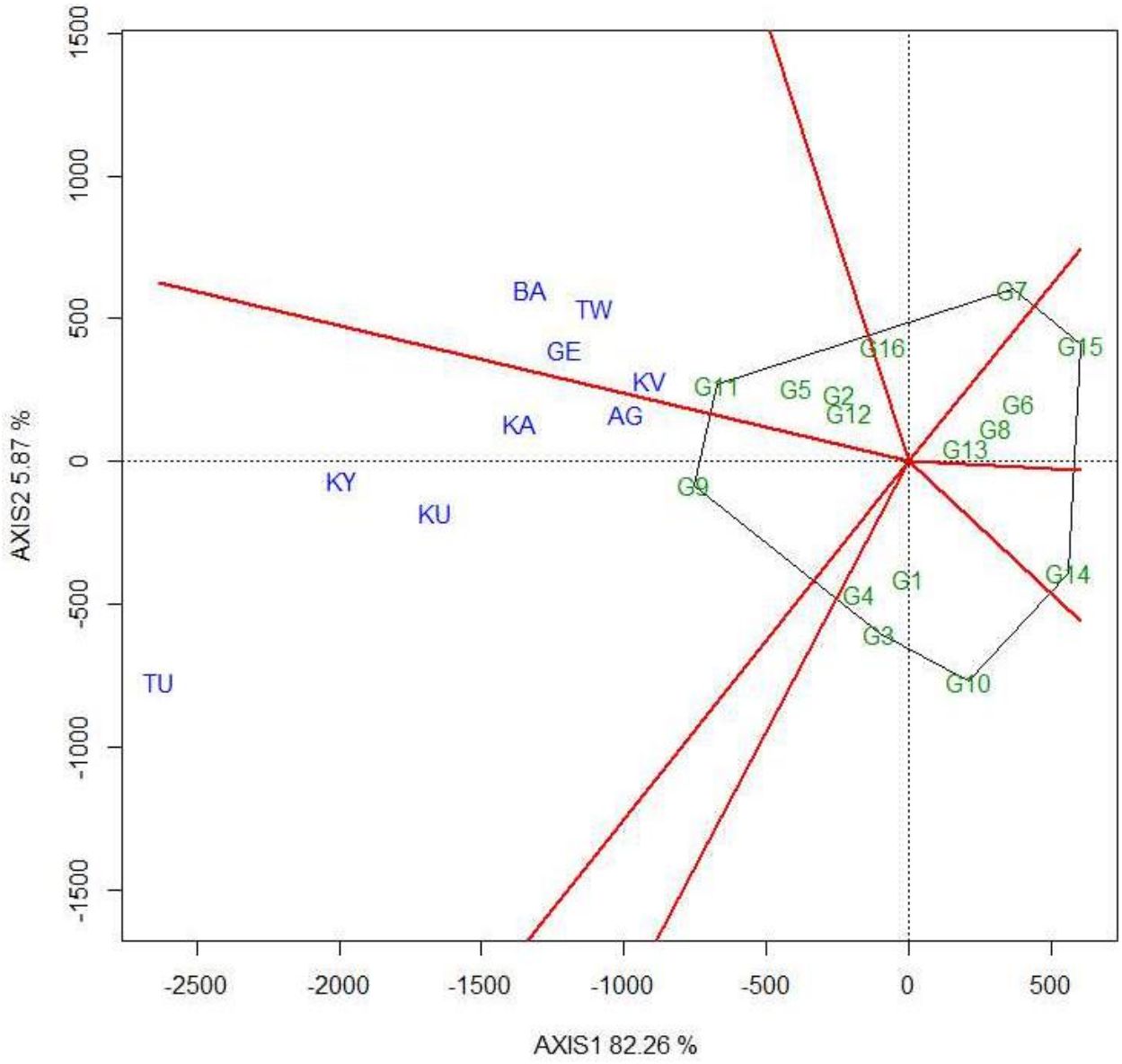


Figure 2.8: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

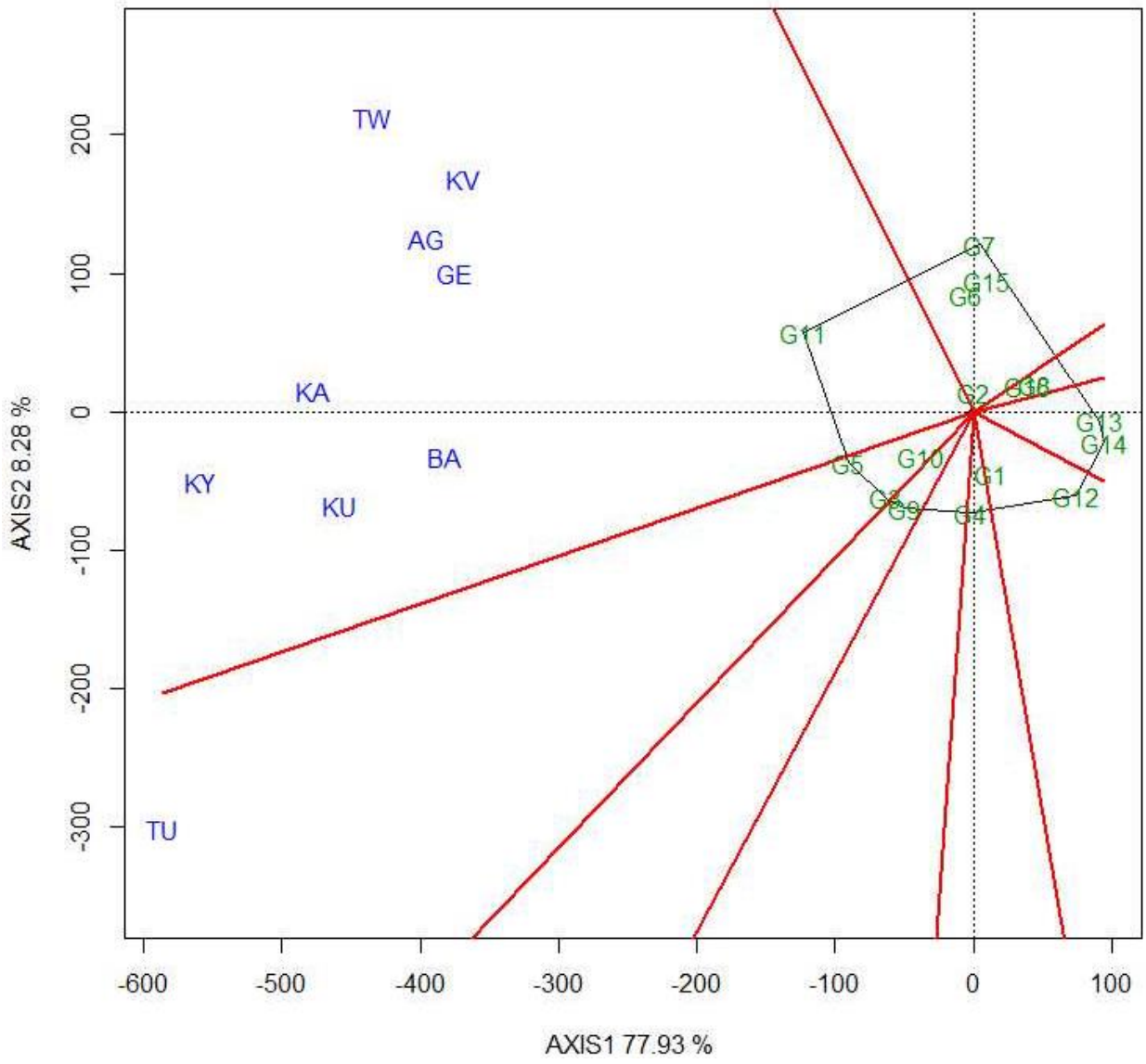


Figure 2.9: The polygon (which-won-where) view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated in two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 2. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

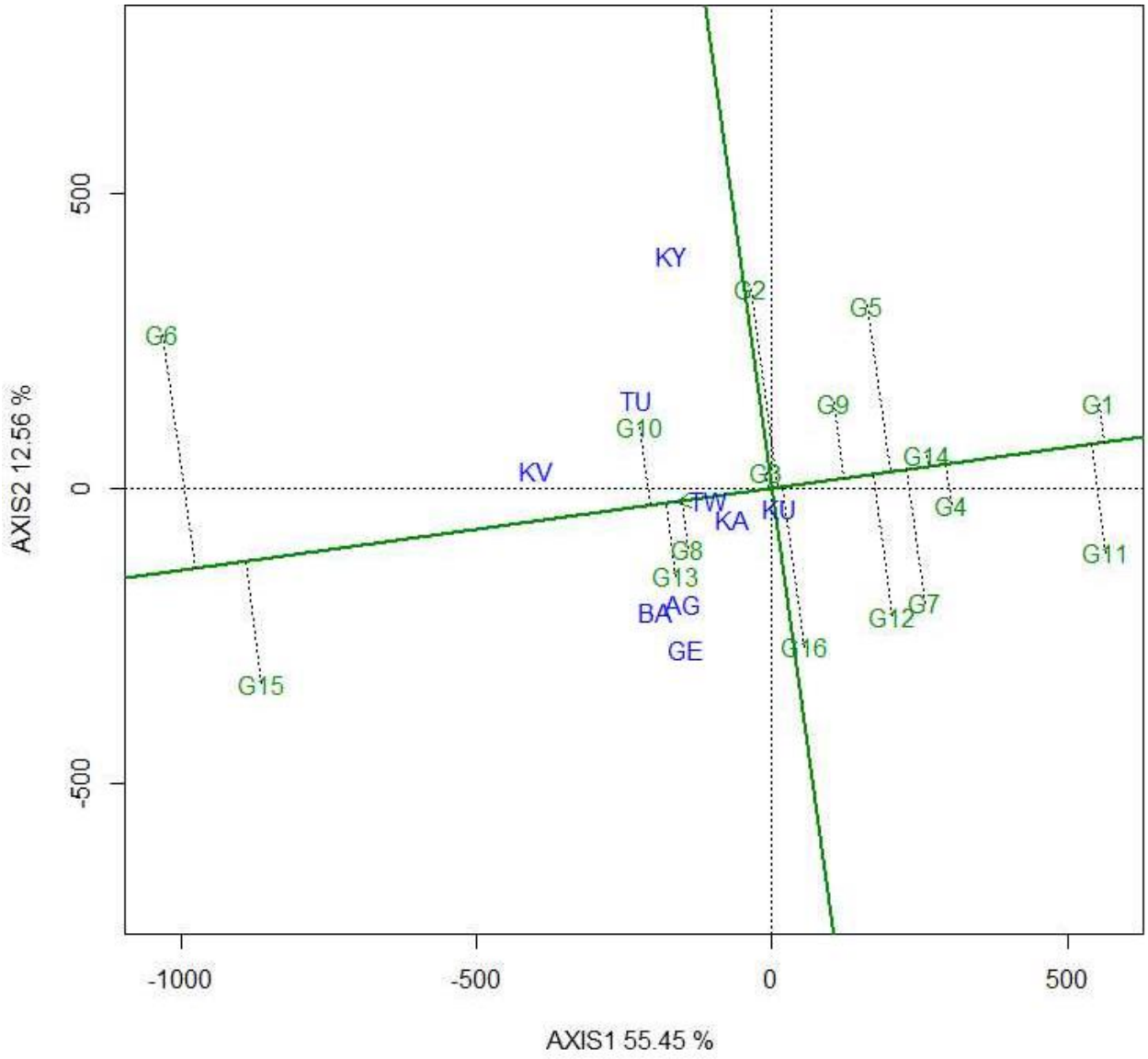


Figure 2.10: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

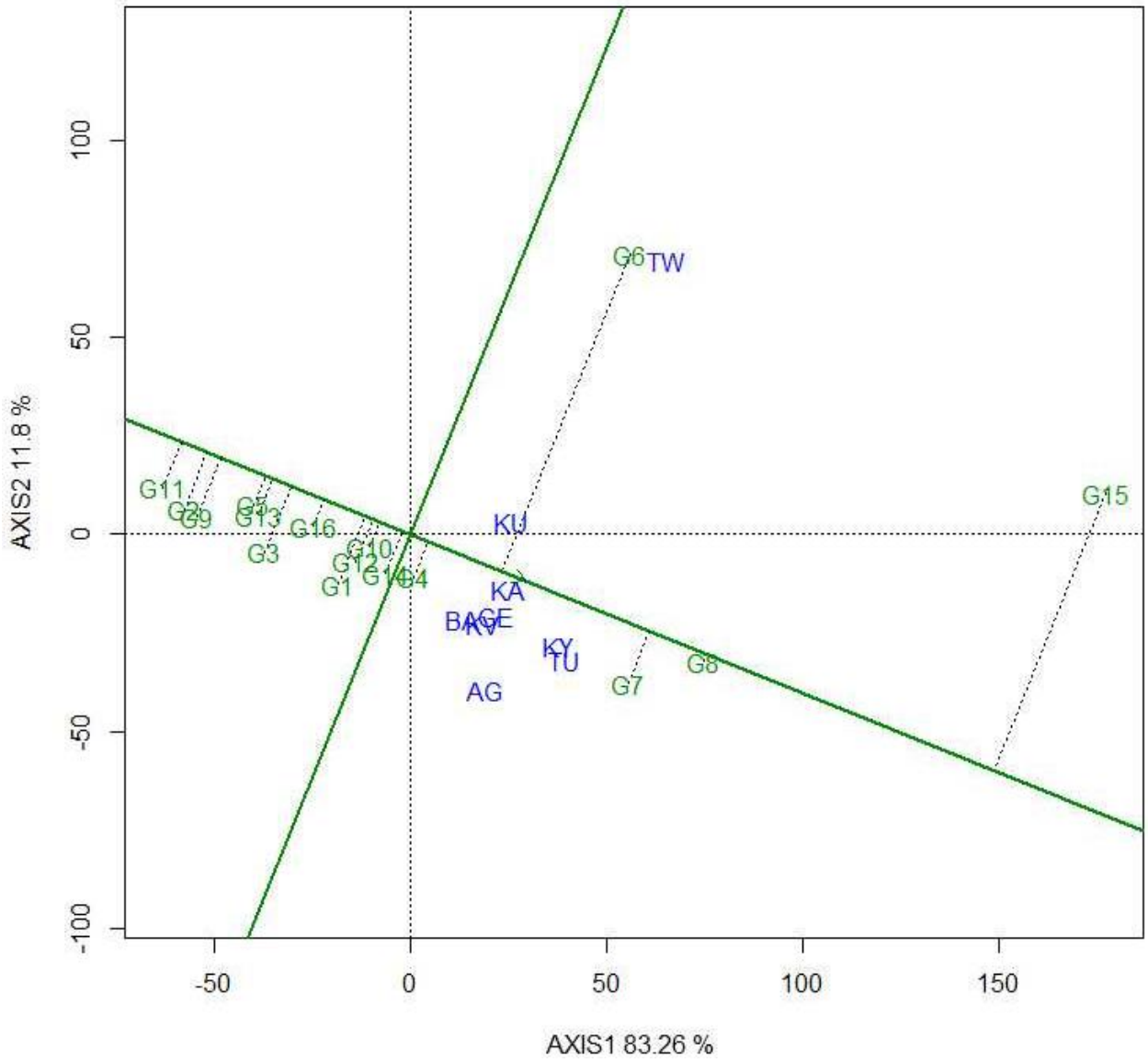


Figure 2.11: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

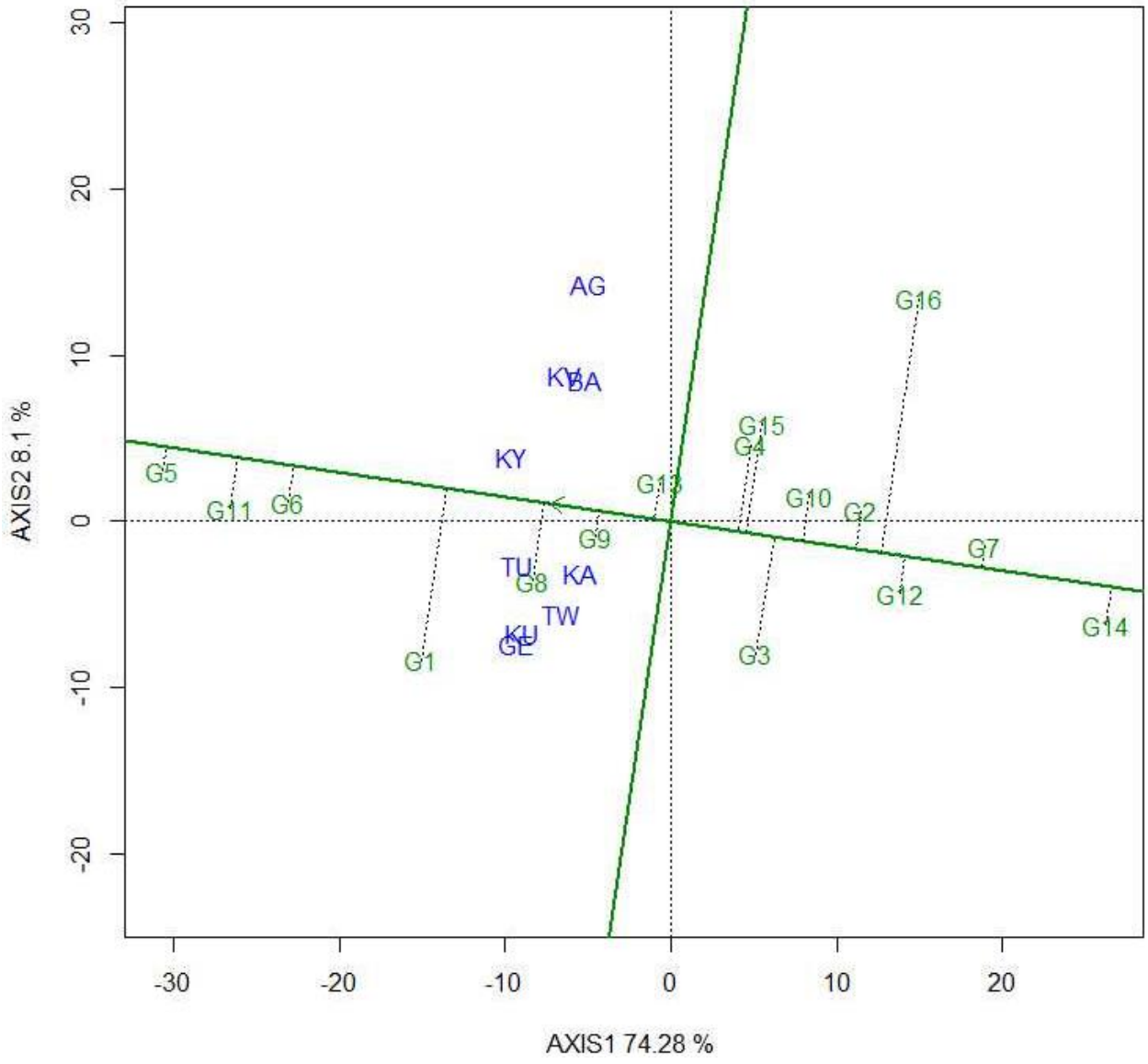


Figure 2.12: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

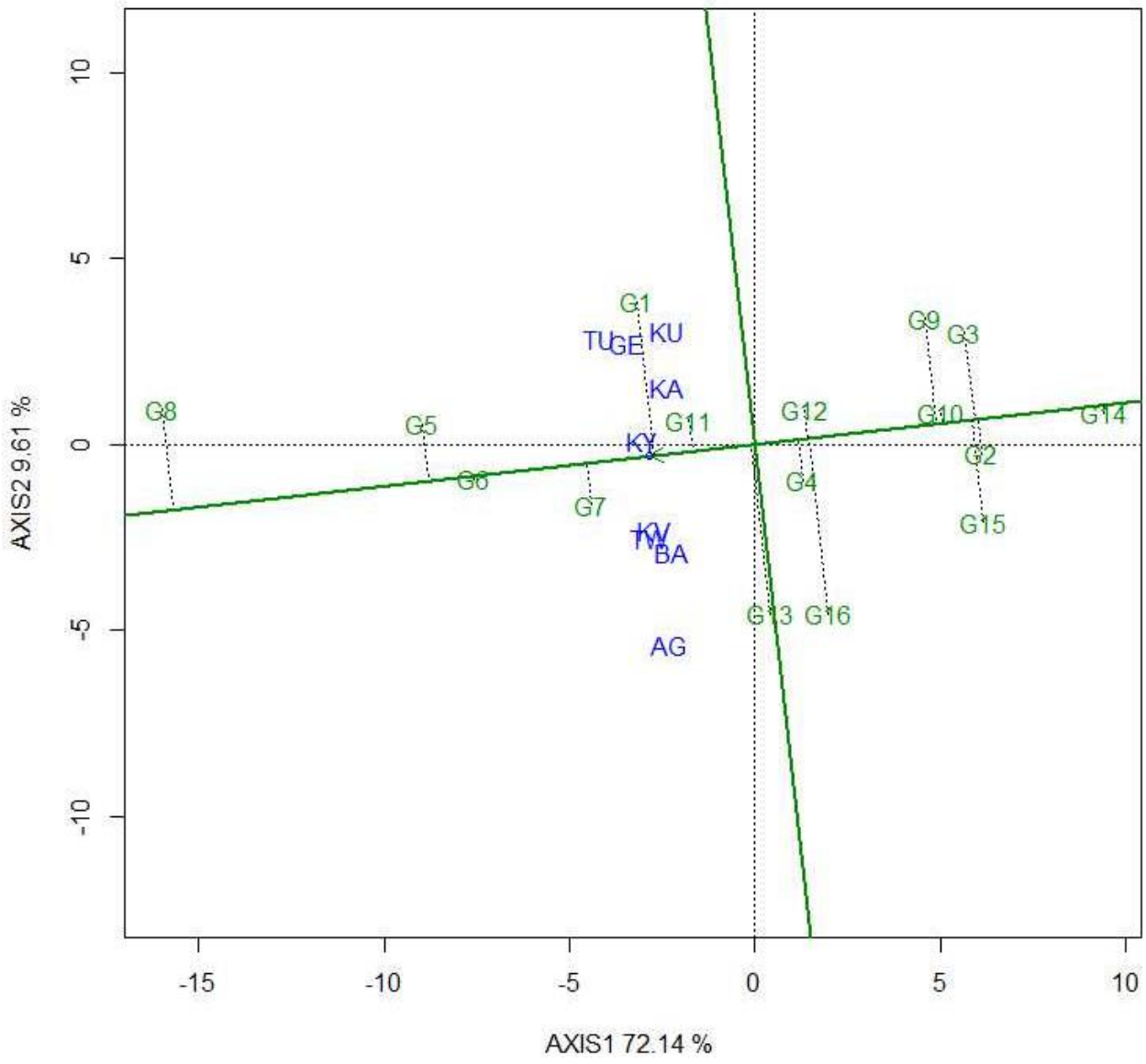


Figure 2.13: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

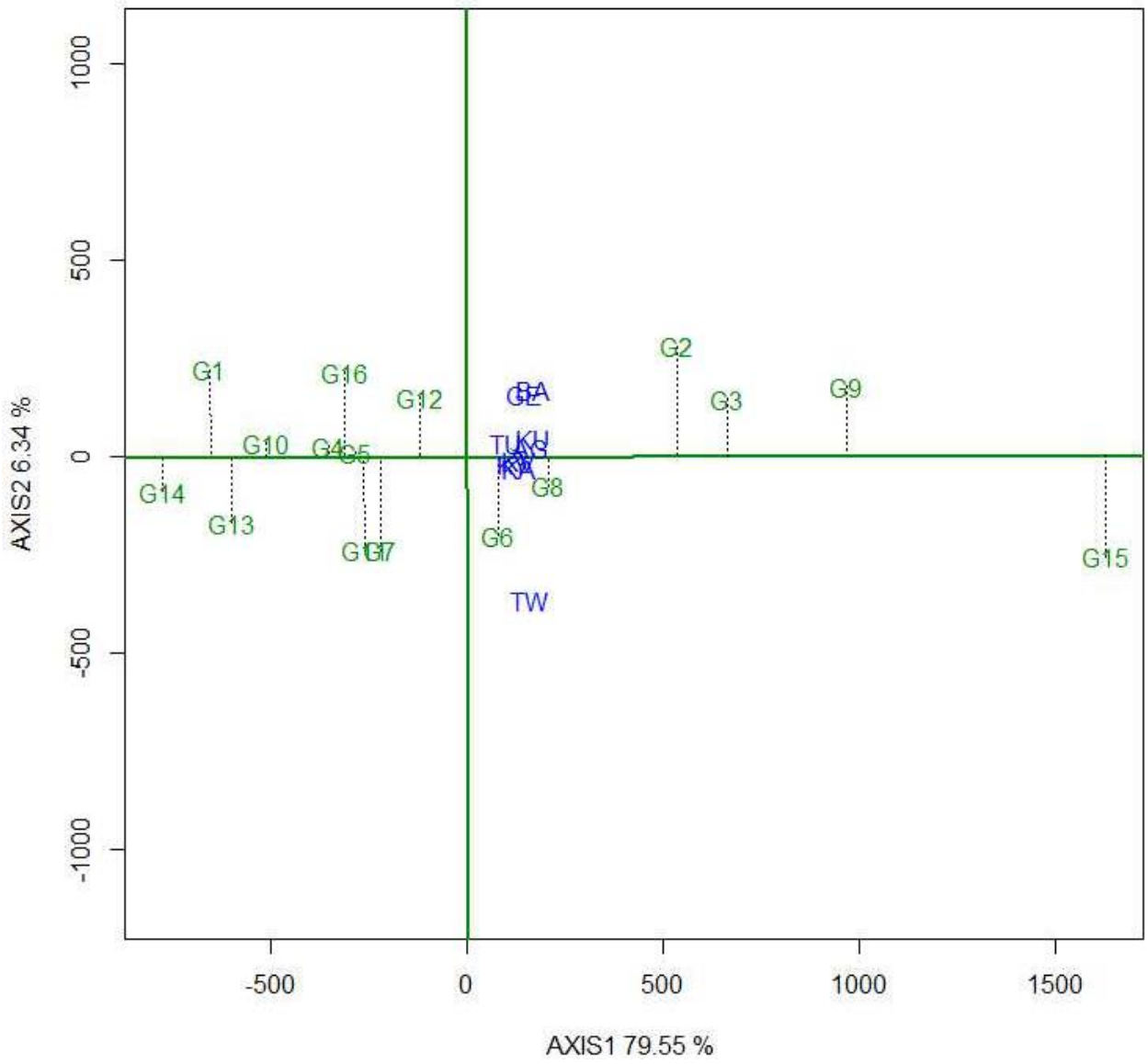


Figure 2.14: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

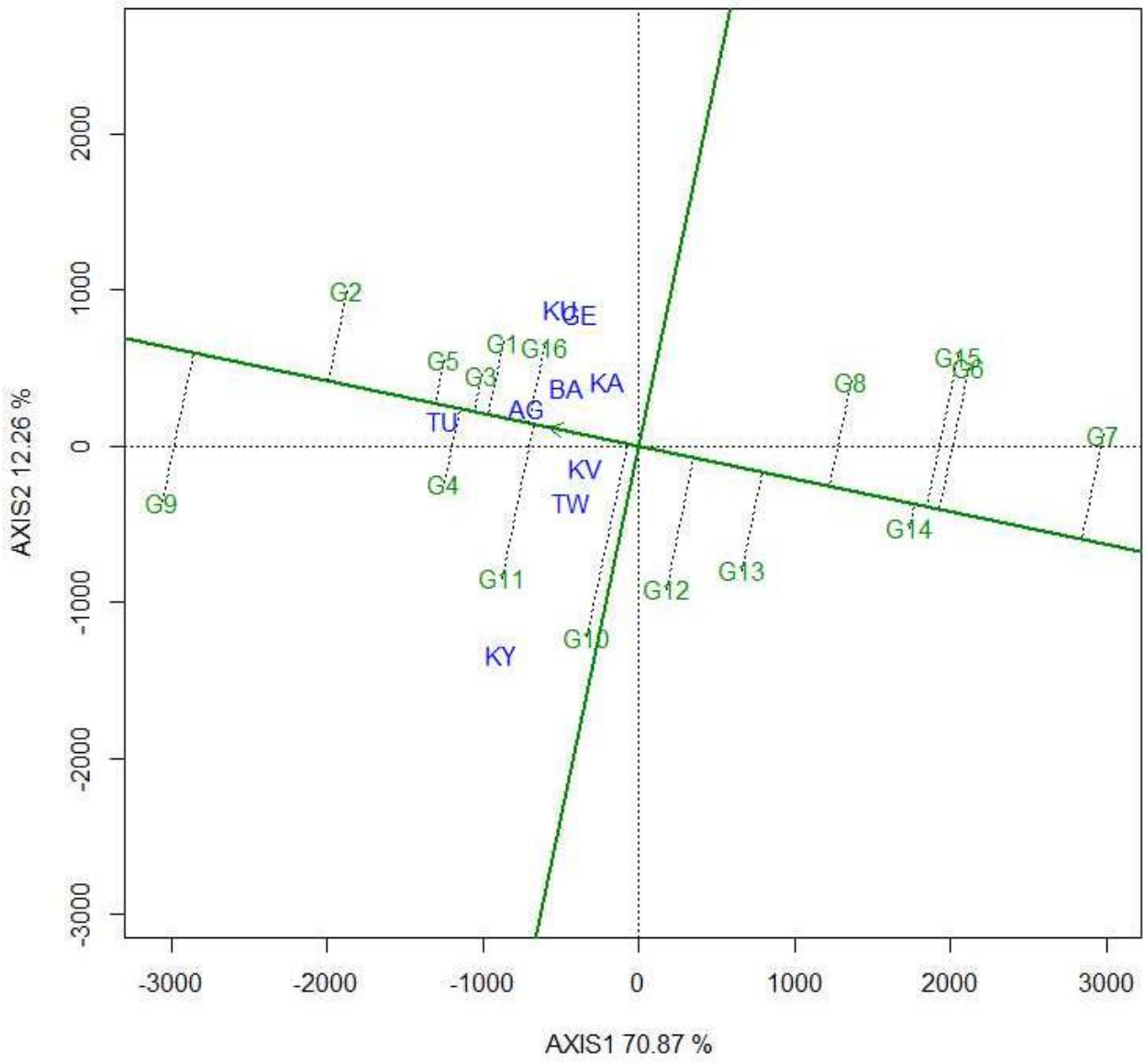


Figure 2.15: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

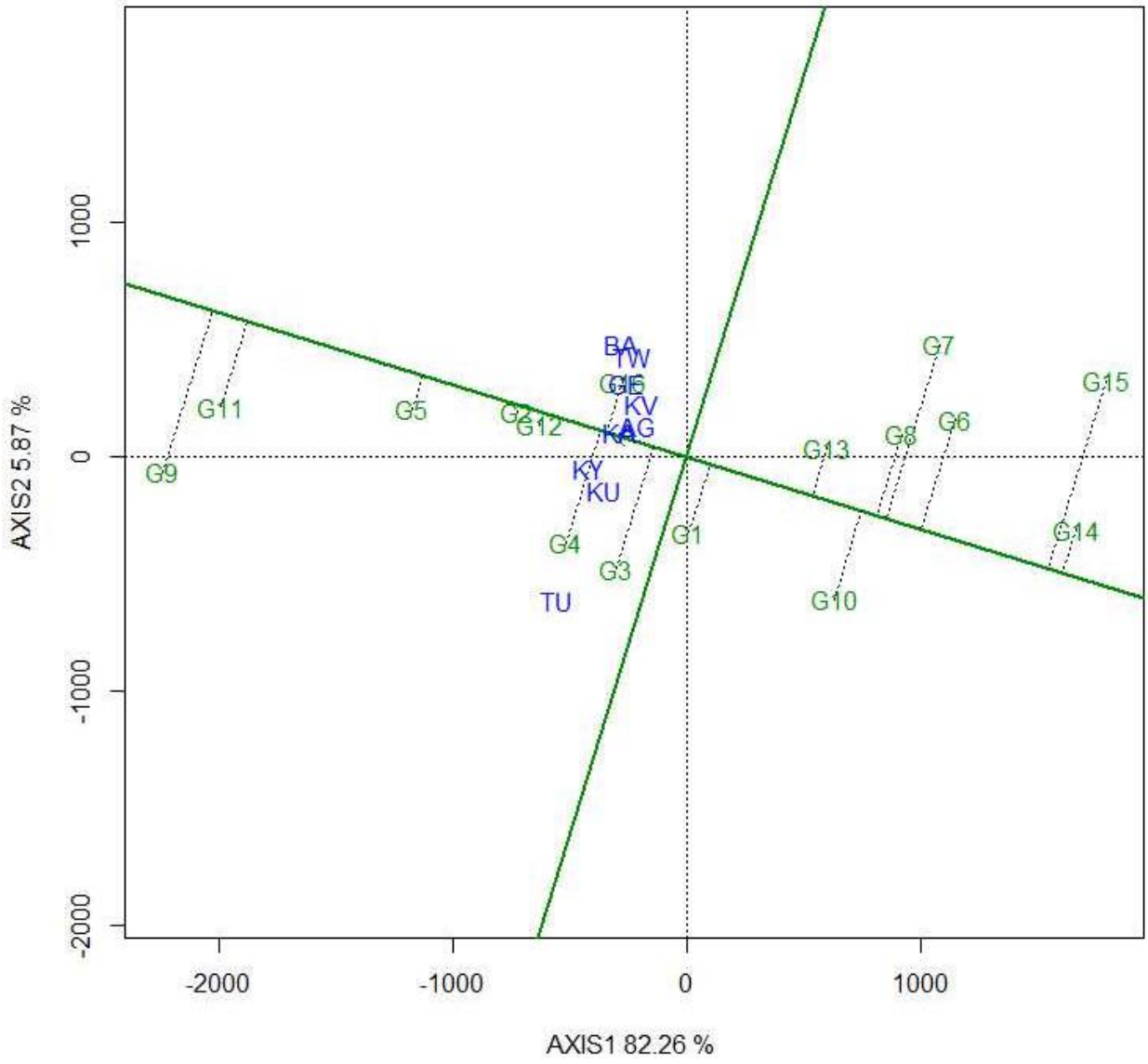


Figure 2.16: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

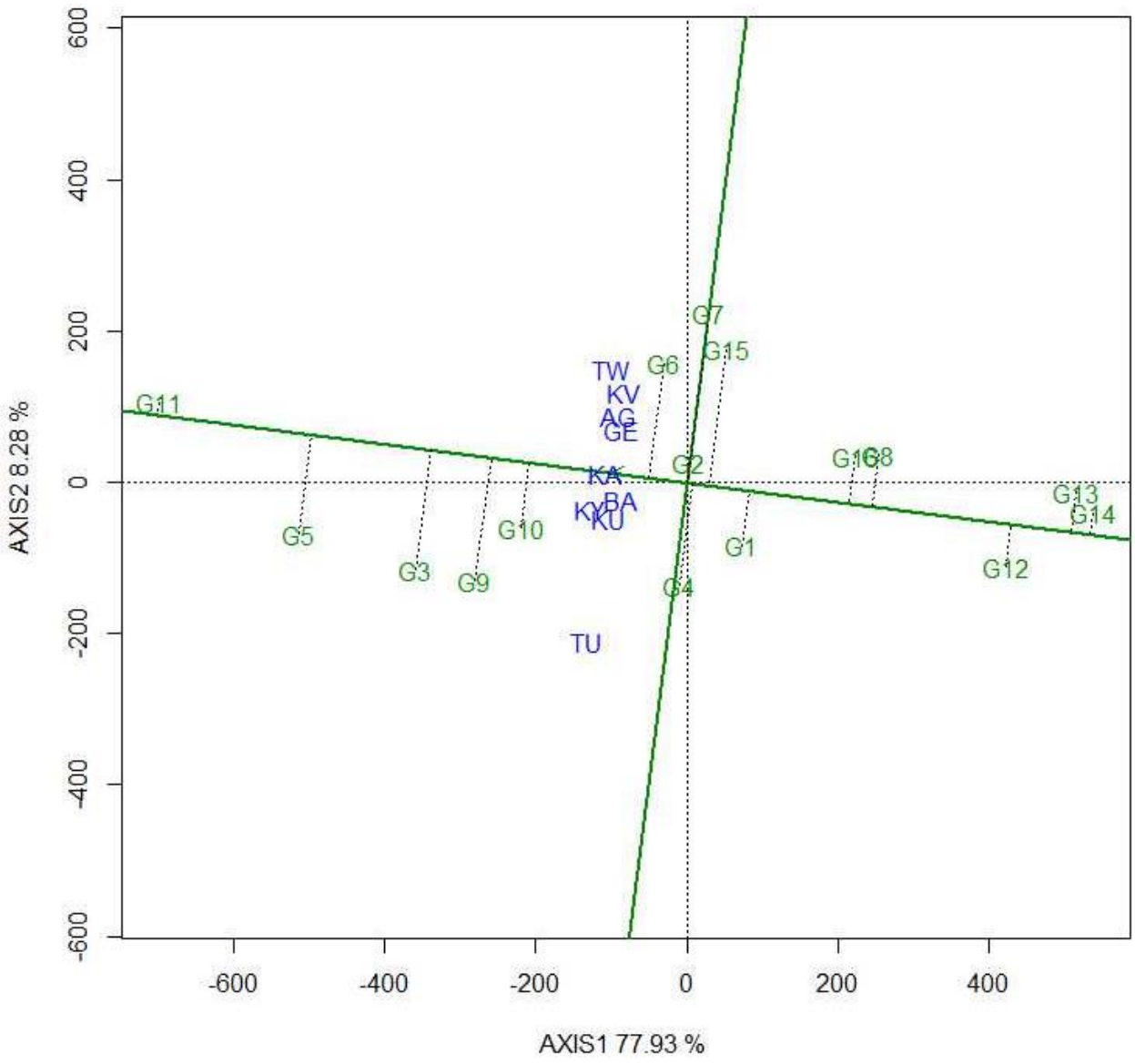


Figure 2.17: Mean performance vs. stability view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

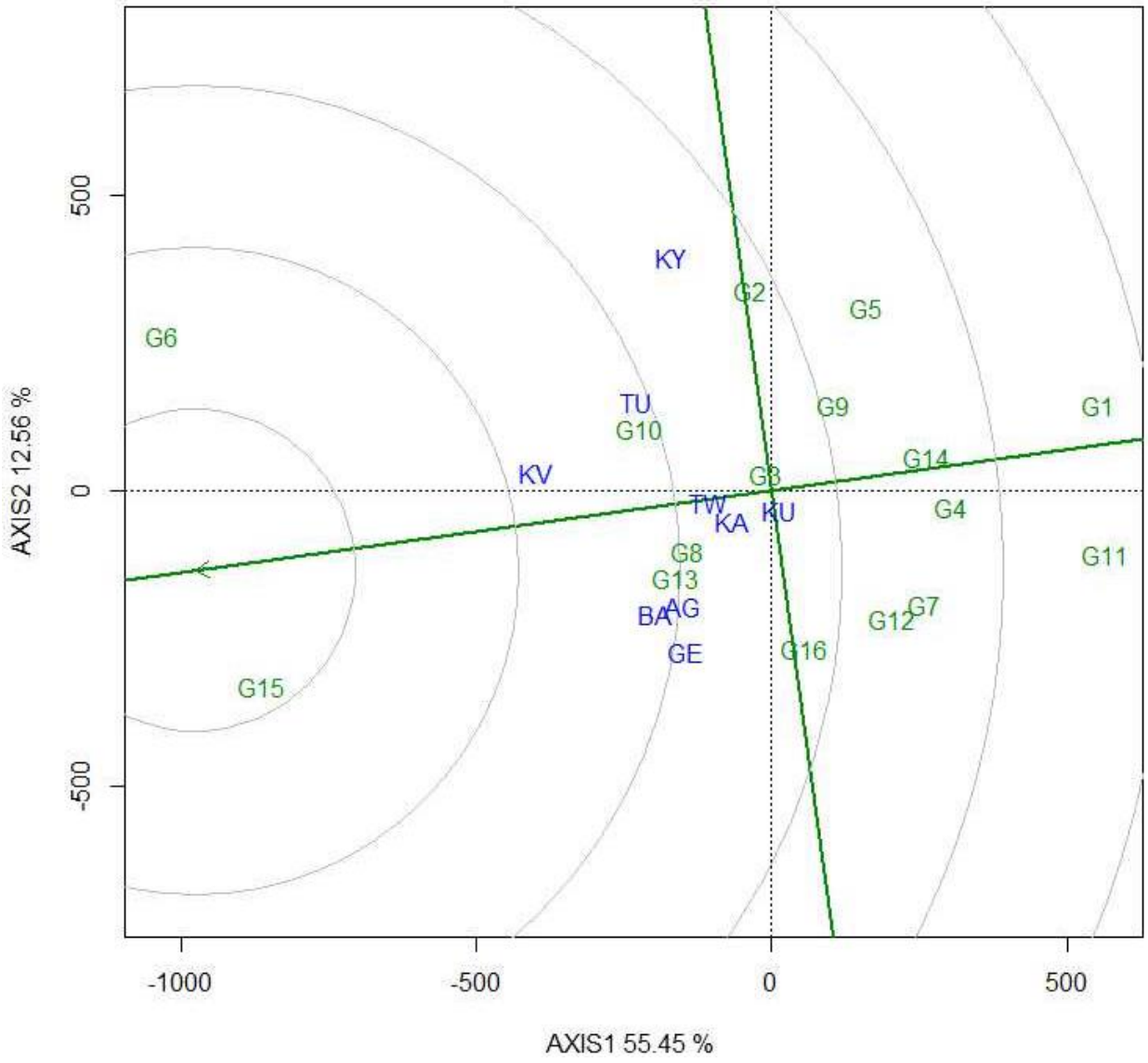


Figure 2.18: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed yield. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

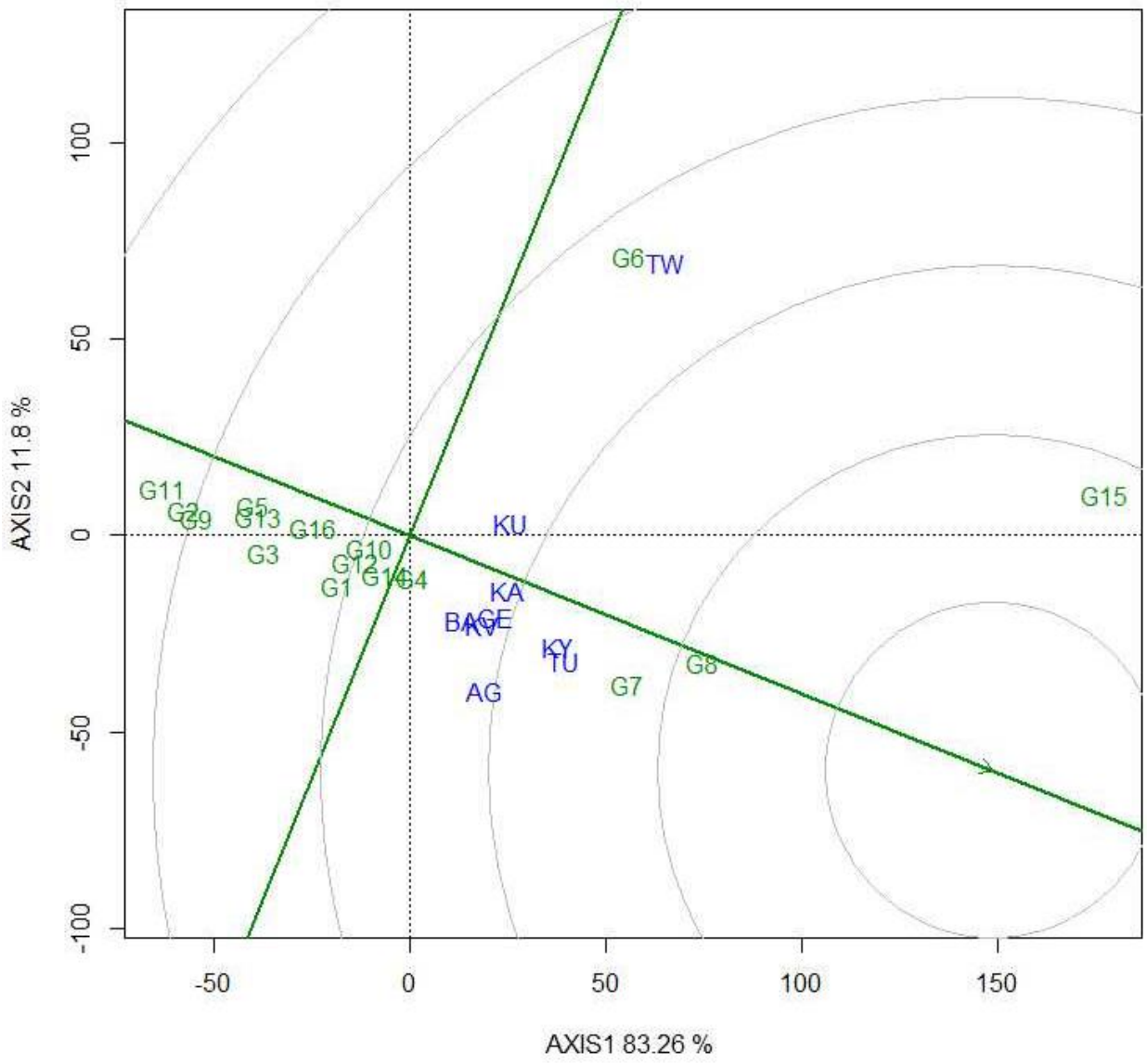


Figure 2.19: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for cooking time. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

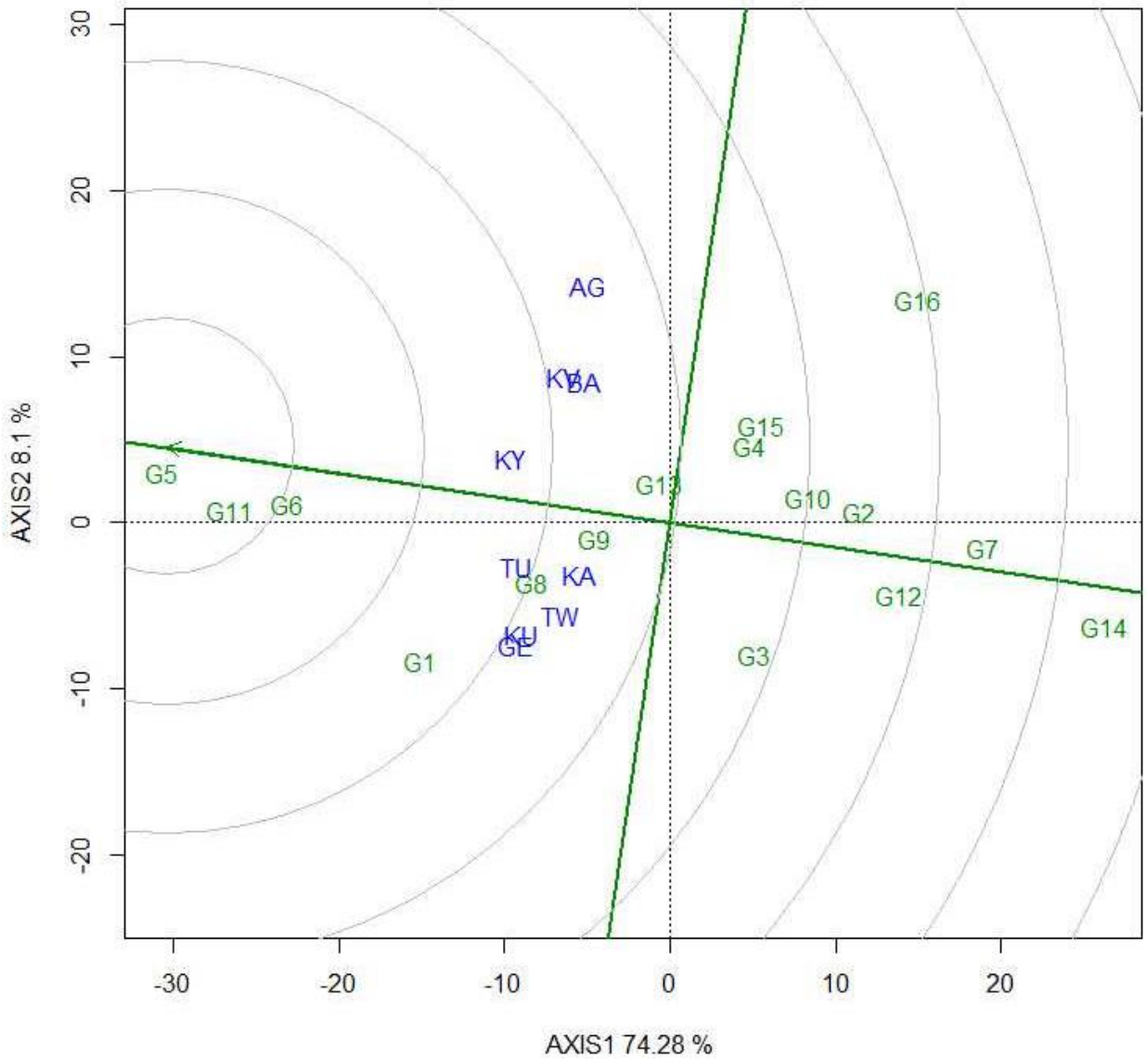


Figure 2.20: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed iron concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

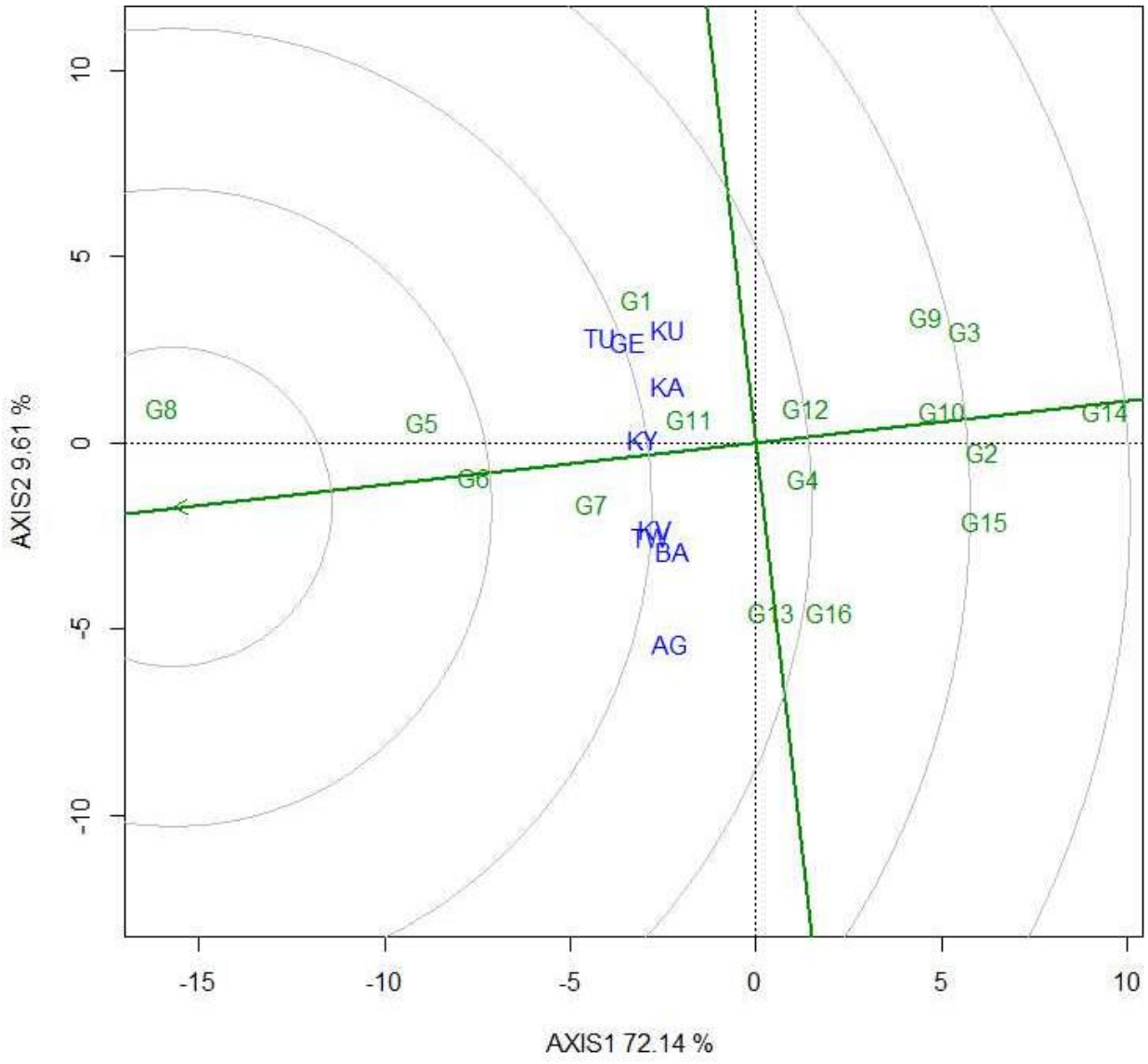


Figure 2.21: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed zinc concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

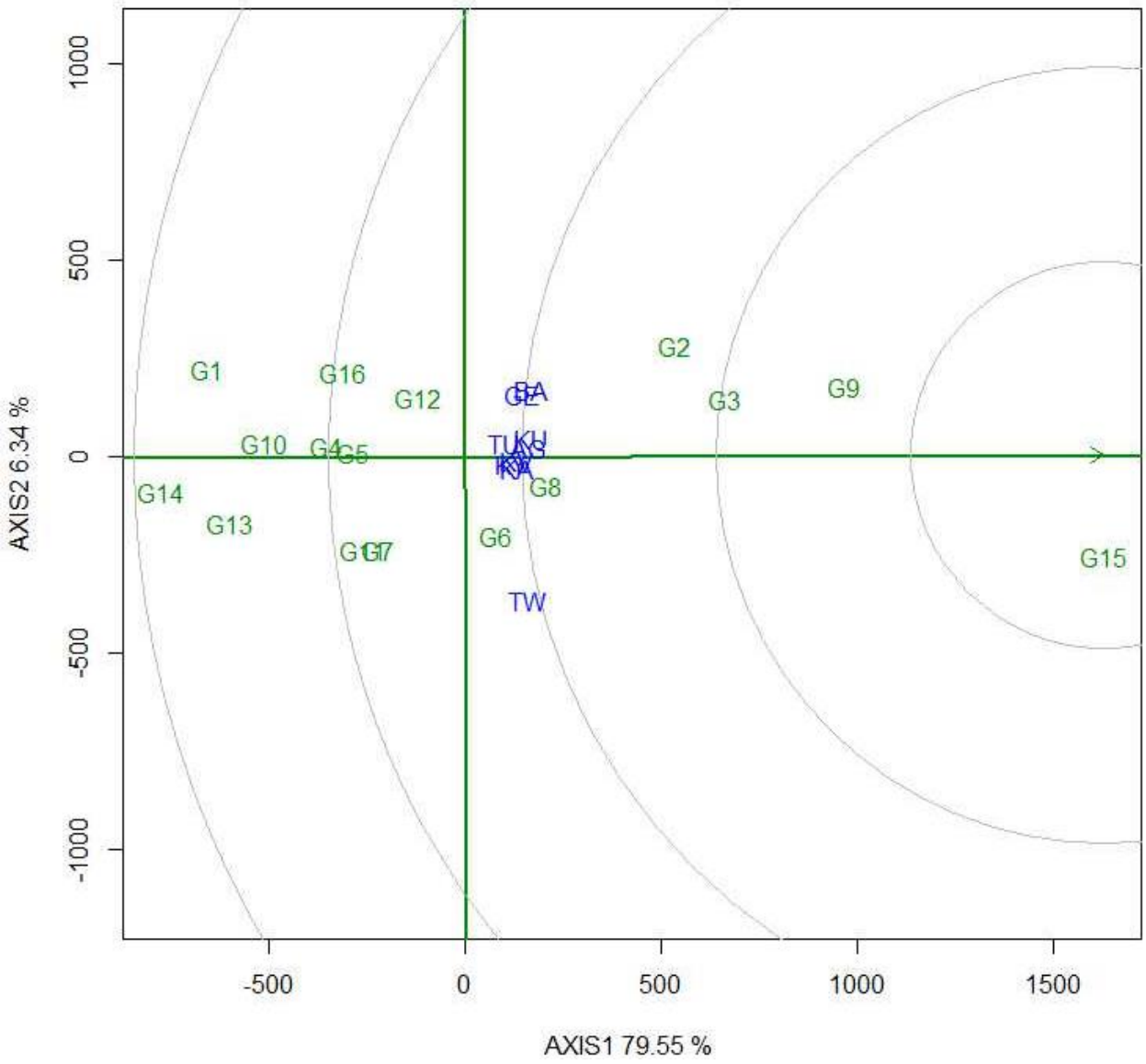


Figure 2.22: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed calcium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

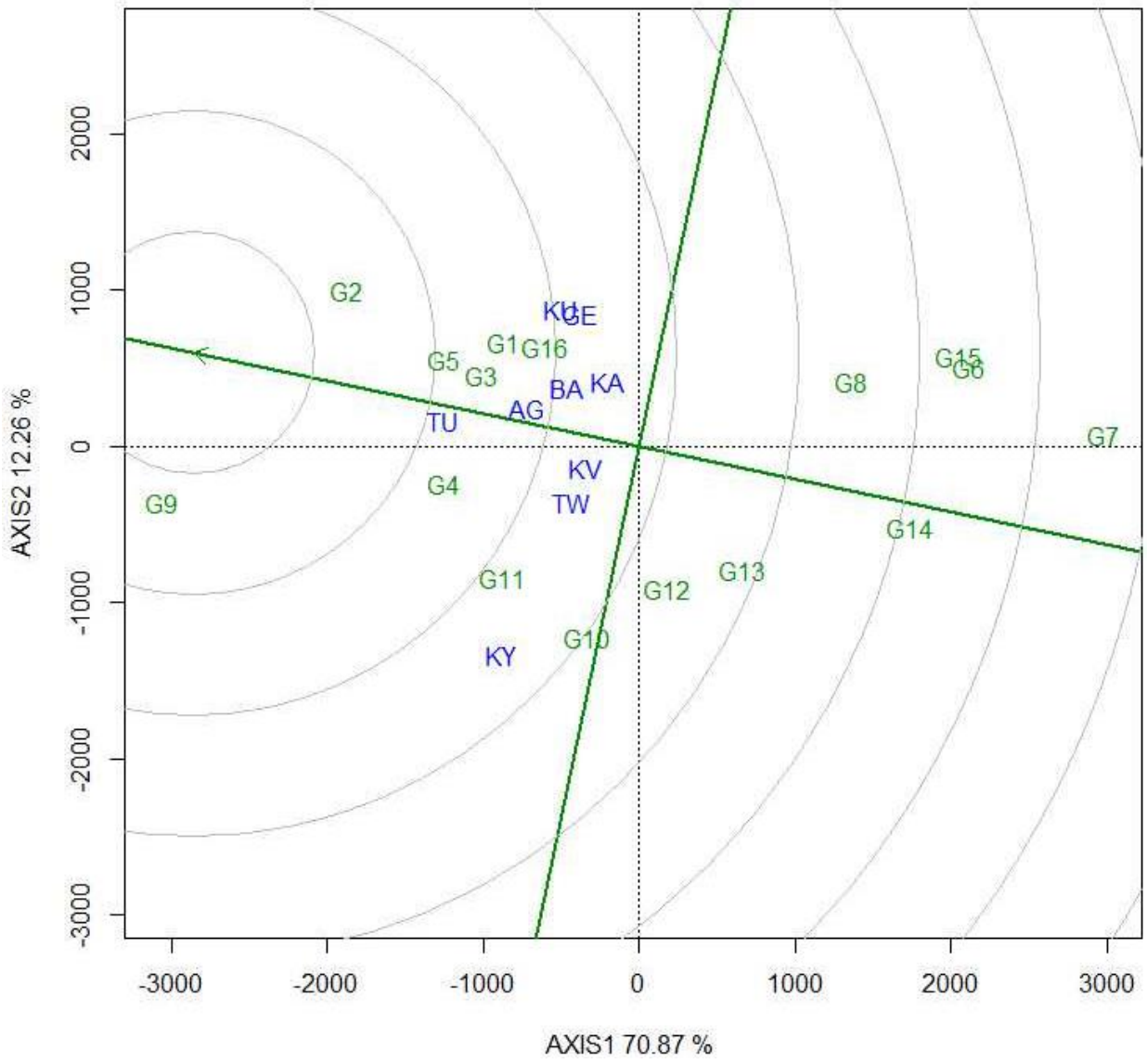


Figure 2.23: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed potassium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

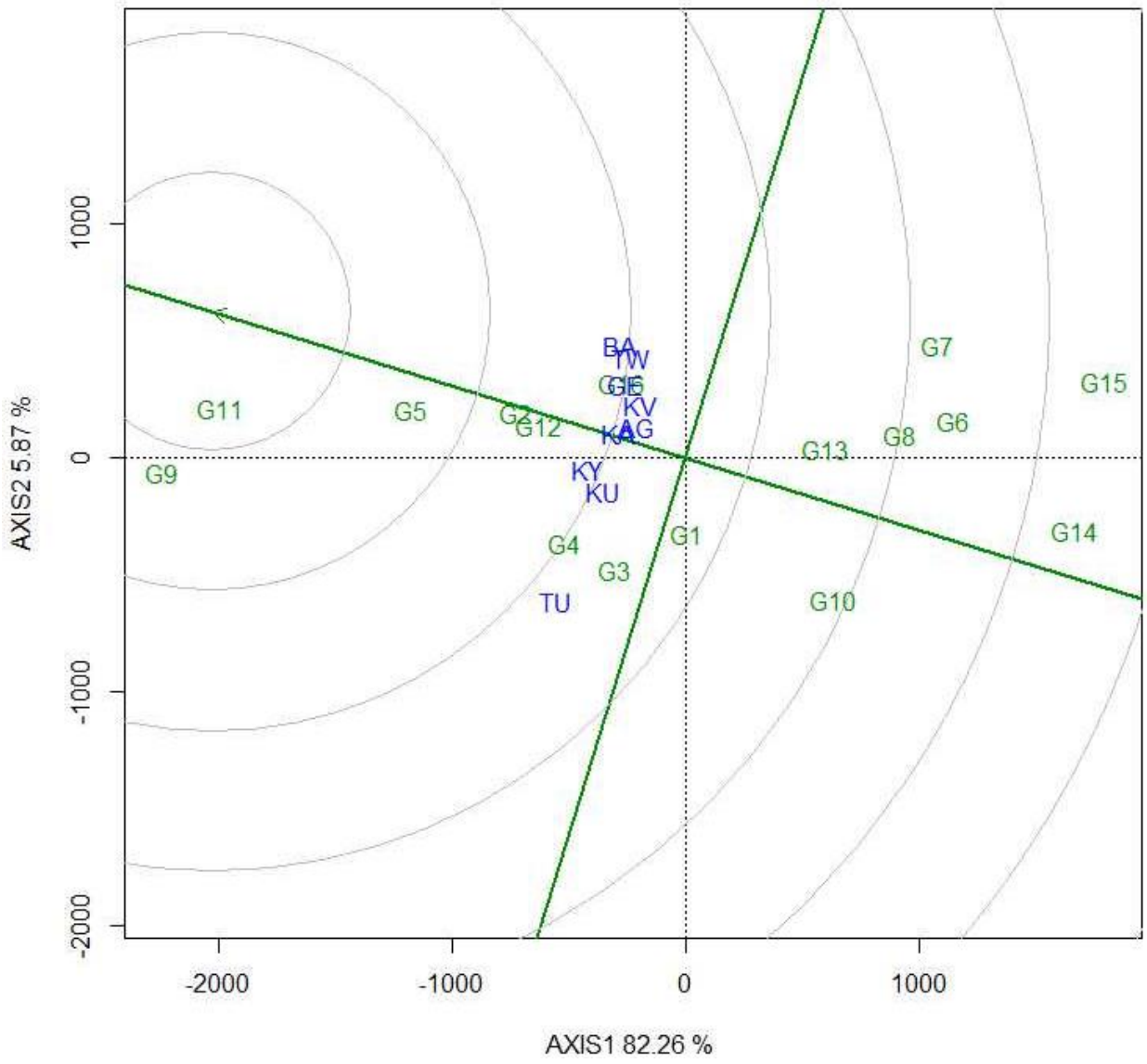


Figure 2.24: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed phosphorus concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

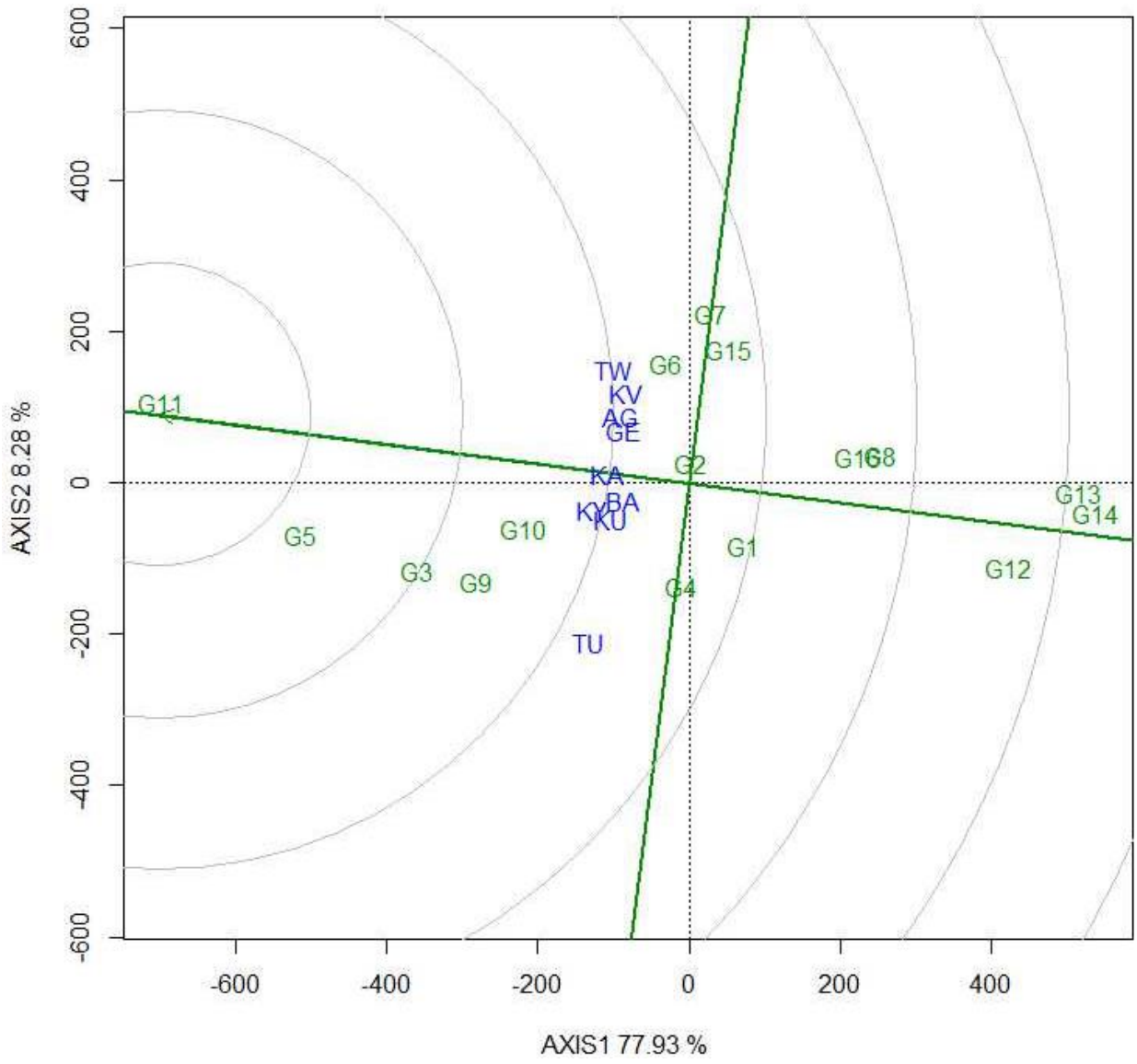


Figure 2.25: The genotype rankings with reference to the ideal genotype view of genotype main effects plus genotype x environment interaction effect (GGE) biplot of 16 common bean genotypes evaluated for two years across nine locations for seed magnesium concentration. The biplots were generated based on a Scaling = 0, Centering = 2, and SVP = 1. The ideal genotype is located inside the innermost concentric circle at the arrowhead of the average environment coordinate (AEC)-abscissa which passes through the biplot origin. Key to the labels of genotypes and locations is presented in Tables 2.1 and 2.3 respectively.

LITERATURE CITED

LITERATURE CITED

Akibode, C.S., Maredia, M. 2011. Global and regional trends in production, trade and consumption of food legume crops. Report submitted to the Standing Panel on Impact Assessment (SPIA) of the CGIAR Science Council, FAO, Rome.

Ali, M.A., 1950. Genetics of resistance to common bean mosaic virus in the bean (Phaseolus vulgaris L.). Phytopathogy 40:69-79.

Alexandratos, N., Bruinsma, J., 2012. World agriculture towards 2030/2050: The 2012 revision. ESA Working paper No. 12-03. Food and Ag. Org. United Nations, Rome.

Annicchiarico, P., Bellah, F., Chiari, T., 2005. Defining sub-regions and estimating the benefits of for a specific-adaptation strategy by breeding programs: A case study. Crop Sci. 45:1741-1749.

Awio, B., Mukankusi, C.M., Nkalubo, S.T., Gibson, P., Malinga, M.G, Rubaihayo, P.R., Edema, R., 2017. Variety x Environment interaction of diseases and yield in selected common bean varieties. Agron. J. 109:2450-2462.

Balakrishnan, D., Subrahmanyam, D., Badri, J., Raju, A.K., Rao, Y.V., Beerelli, K., Mesapogu, S., Surapaneni, M., Ponnuswamy, R., Padmavathi, G., Babu, V.R., Neelamraju, S., 2016. Genotype x environment interactions of yield traits in backcross introgression lines derived from Oryza sativa cv Swarna/Oryza nivara. Front. Plant Sci. 7:1530.

Bailey, R.L., West Jr, K.P., and Black, R.E., 2015. The epidemiology of global micronutrient deficiencies. Ann. Nutr. Metab. 66:22-33.

Baxter, I., 2015. Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J. Exp. Bot. 66:2127-2131.

Baxter, I., Hermans, C., Lahner, B., Yakubova E., Tikhonova, M., Verbruggen, N., Chao, D., Salt, D.E., 2012. Biodiversity of mineral nutrient and trace element accumulation in Arabidopsis thaliana. PLoS One. 7:e35121.

Beaver, J.S., Kelly, J.D., 1994. Comparison of selection methods for dry bean populations derived from crosses between gene pools. Crop Sci. 25:923-926.

Birol, E., Meenakshi, J.V., Oparinde, A., Perez, S., Tomlins, K., 2015. Developing country consumers' acceptance of biofortified foods: a synthesis. Food Secur. 7:555-568.

Blair, M.W., 2013. Mineral biofortification strategies for food staples: The example of common bean. J. Agric. Food Chem. 61:8287-8294.

Bucheyeki, T.L., Mmbaga, T.E., 2013. On-Farm Evaluation of Beans Varieties for Adaptation and Adoption in Kigoma Region in Tanzania. ISRN Agron. Article ID 436064. http://dx.doi.org/10.1155/2013/436064

Checa, O.E., Blair, M.W., 2012. Inheritance of yield related traits in climbing beans (*Phaseolus vulgaris* L.). Crop Sci. 52:1998-2013.

Chowdhury, S., Meenakshi, J.V., Tomlins, K., Owori, C., 2011. Are consumers in developing countries willing to pay more for micronutrient-dense biofortified foods? Evidence from a field experiment in Uganda. Am. J. Agric. Econ. 93:83-97.

Cichy, K.A., Wiesinger, J.A., Mendoza, F.A., 2015. Genetic diversity and genome-wide association analysis of cooking time in dry bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 128:1555-1567.

Frutos, E., Galindo, M.P., Leiva, V., 2014. An interactive biplot implementation in Rfor modeling genotype-by-environment interaction. Stochastic Environ. Res. Risk Assess. 28:1629-1641.

Gibson, R., Ferguson, E., 2008. An interactive 24 hour recall for assessing the adequacy of iron and zinc intakes in developing countries. Washington (DC): HarvestPlus; 2008.

Gyawali, S., Sunwar, S., Subedi, M., Tripathi, M., Joshi, K.D., and Witcombe, J.R., 2007. Collaborative breeding with farmers can be effective. Field Crops Res. 101:88-95.

Hailu, N., Fininsa, C., Tana, T., Mamo, G., 2017. Effects of temperature and moisture on growth of common bean and its resistance reaction against common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli* strains). J. Plant Pathol. Microbiol. 8:419.

Hotz, C., Abdelrahman, L., Sison, C., Moursi, M., Loechl, C., 2011. A food composition table for central and eastern Uganda. Washington (DC): HarvestPlus; 2011.

Schoonhoven, A., Pastor-Corrales, M.A., (comps.). 1987. Standard system for the evaluation of bean germplasm. Centro Internacional de Agricultura Tropical (CIAT), Cali, CO. 56 p.

Tumwegamire, S., Rubaihayo, P.R., Gruneberg, W.J., LaBonte D.R., Mwanga R.O.M., Kapinga, R., 2016. Genotype x environment interactions for East African orange-fleshed sweet potato clones evaluated across varying eco-geographic conditions in Uganda. Crop Sci. 56:1628-1644.

Kelly, J.D., Afanador, L., Haley, S.D., 1995. Pyramiding genes for resistance to bean common mosaic virus. Euphytica. 82:207-212.

Kelly, J.D., Kolkman, J.M., Shneider, K., 1998. Breeding for yield in drybean (*Phaseolus vulgaris* L.). Euphytica 102:343-356.

Kimbugwe, K., 2013. Three year production sector development plan. Rakai district local government. Production and marketing department. Kampala.

Kiwuka, C., Bukenya, Z.R., Namaganda, M., Mulumba, J.W., 2012. Assessment of common bean cultivar diversity in selected communities of central Uganda. Afr. Crop Sci. J. 20:149-158.

Laurie S.M., Booyse, M., Labuschagne, T., Greyling, M.M., 2015. Multienvironment performance of new orange-fleshed sweet potato cultivars in South Africa. Crop Sci. 55:1585-1595.

Manzeke G.M., Mapfumo, P., Mtambanengwe, F., Chikowo, R., Tendayi T., Cakmak, I., 2012. Soil fertility management effects on maize productivity and grain zinc content in smallholder farming systems of Zimbabwe. Plant Soil. 361:57-69.

Masinde, E.M., Mkamillo, G., Ogendo, J.O., Hillocks, R., Mulwa, R.M.S., Kimata, B., Maruthi, M.N., 2018. Genotype by environment interactions in identifying cassava (*Manhot esculenta* Crantz) resistant to cassava brown streak disease. Field Crops Res. 215:39-48.

McClean, P.E., Moghaddam, S.M., Lopez-Millan, A., Brick, M.A., Kelly, J.D., Miklas, P.N., Osorno, J., Porch, T.G., Urrea C.A., Soltani, A., Grusak, M.A., 2017. Phenotypic diversity for seed mineral concentration in North American dry bean germplasm of middle American ancenstry.

Mubiru, D.N., Kyazze, F.B., Radeny, M., Zziwa, A., Lwasa, J., Kinyangi, J., 2015. Climatic trends, risk perceptions, and coping strategies of smallholder famrers in rural Uganda. Working paper, CGIAR Research Program on Climate Change, Agriculture and Food Security. 23 June 2015. Climate Change, Agric. and Food Sci., Copenhagen, Denmark. www.ccafs.cgiar.org. (Accessed on 1st November 2017).

Nziguheba, G., 2007. Overcoming phosphorus deficiency in soils of Eastern Africa: recent advances and challenges. In: Bationo, A., Waswa, B., Kihara, J., Kimetu, J., (eds) Advances in integrated soil fertility management in sub-Saharan Africa: challenges and opportunities. Springer, New York, pp 49-160.

Okii, D., Tukamuhabwa, P., Kami, J., Namayanja, A., Paparu, P., Ugen, M., Gepts, P., 2014. The genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) germplasm in Uganda. Afr. J. Biotech. 13:2935-2949.

Olango, N., Tusiime, G., Mulumba, J.W., Nankya, R., Fadda, C., Jarvis, I.D., Paparu, P., 2017 Response of Ugandan common bean varieties to Pseudocercospora griseola and Angular leaf spot disease development in varietal mixtures. Int. J. Pest Manag. 2017, 63:119.127.

Pyndji, M.M., Trutmann, P., 1992. Managing angular leaf spot on common bean in Africa by supplementing farmer mixtures with resistant varieties. Plant Dis. 76:1144-1147

SAS Institute., 2011. SAS Version 9.4 SAS Institute Inc., Cary, NC, USA.

Sibiko, K.W., Ayuya, O.I., Gido, E.O., Mwangi, J.K., 2013. An analysis of economic efficiency in bean production: evidence from Eastern Uganda. J. Econ. Sustain. Dev. 4:1-9.

Silbernagel, M.J., Mills, L.J., Wang, W.Y., 1986. Tanzanian strain of bean common mosaic virus. Plant Dis. 70:839-841.

Singh, S.P., Teran, H., Munoz, C.G., Orsono, J.M. 2002. Selection for seed yield in Andean intra-gene pool and Andean x Middle American inter-gene pool populations of common bean. Euphytica. 127:437-444.

Tokatlidis, I.S., Papadopoulos, I.I., Baxevanos, D., Koutita, O., 2010. Genotype x environment effects on single plant selection at low density for yield and stability in climbing dry bean populations. Crop Sci. 50:775-783.

Uganda Bureau of Statistics., 2010. Uganda Census of Agriculture 2008/2009. Crop area and production report, vol IV, p 178.

Yan, W., 2002. Singular-value partition for biplot analysis of multi-environment trial data. Agron. J. 94:990-996.

Yan, W., Fregeau-Reid, J., 2008. Breeding line selection based on multiple traits. Crop Sci. 48:417-423.

Yan, W., Hunt, L.A, Sheng, Q., Szlavnics, Z., 2000. Cultivar evaluation and mega-environment investigation based on GGE biplot. Crop Sci. 40:597-605.

Yan, W., Kang, M.S., Ma, B., Woods, S., Cornelius, P.L., 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci. 47:643-653.

Yan, W., Tinker N.A. 2006. Biplot analysis of multi-environment data: Principles and applications. Can. J. Plant Sci. 86:623-645.

Yan, W., Tinker N.A. 2005. An integrated biplot analysis system for displaying, interpreting, and exploring genotype x environment interaction. Crop Sci. 45:1004-1016.

Webb, P., Kennedy, E., 2014. Impacts of agriculture on nutrition: nature of the evidence and research gaps. Food Nutr. Bull. 35:126-32.

CHAPTER 3

IDENTIFICATION OF FARMERS' PRIORITIES FOR HIGH MINERAL AND FAST COOKING DRY BEANS (*Phaseolus vulgaris* L.) THROUGH PARTICIPATORY VARIETY SELECTION AND SENSORY EVALUATION IN UGANDA

Identification of farmers' priorities for high mineral and fast cooking dry beans (*Phaseolus vulgaris* L.) through participatory variety selection and sensory evaluation in Uganda

Abstract

Common bean (*Phaseolus vulgaris* L.) is the most important food legume crop in Uganda. Beans are rich in fiber, micronutrients, and protein. Most of the research work in common bean breeding in Uganda has focused on developing genotypes with superior agronomic performance with limited focus on nutritional quality traits (micronutrients, cooking time, and sensory attributes). Farmers desire to be involved in variety evaluation and selection and also demand dry bean varieties with a good balance of agronomic, nutrient composition, cooking time, and sensory attributes. A study was designed to understand farmers' priority traits for biofortified beans and determine genotype effects on the sensorial attributes of farmers' selected accessions using the participatory variety selection and consumer sensory evaluation methodologies. The best five genotypes including a local check were selected at harvest by growers based on the positive and negative traits from a set of 16 accessions grown on-farm in Uganda. The selected best five genotypes were then cooked for evaluation by 108 sensory panelists at three central locations. The study showed that over 80 % of the growers want varieties that are early maturing, high yielding, fast cooking, and have high market potential. Over 90 % of the growers and consumers prefer red mottled beans while over 68 % prefer yellow beans followed by white beans at 64 %. About 90 % of the growers prefer flavorful bean varieties. Sensory analysis results showed that the landrace Ervilha had better bean flavor than the local check variety NABE-15 and the landrace PI527538. Both Ervilha and the landrace Chijar tasted better to the sensory panelists compared to the genotype PI527538. Genotype Ervilha had softer texture compared to all the rest of the evaluated bean accessions. The overall acceptance among sensory panelists was higher for Ervilha compared

to the landraces Rozi Koko and PI527538. The identification of germplasm with favorable attributes and uncovering of growers' and consumers' priority traits will help bean breeders in the development of common bean varieties that can effectively meet the needs of growers and consumers alike.

Introduction

Common bean is the most important food legume in Uganda and around the world (Akibode and Maredia, 2012). It is widely grown for its nutrients particularly the high fiber concentration, protein, and minerals like iron and zinc (Beebe, 2012). Dry beans are grown in nearly all districts in Uganda, and are consumed in virtually every household and schools throughout Uganda (Uganda Bureau of Statistics 2010; Broughton et al., 2003). Approximately, 420,000 metric tons of beans are grown each year on 753,000 hectares of land making the crop number five in importance after bananas, maize, sweet potatoes, and cassava (Uganda Bureau of Statistics 2010). Uganda has a high per-capita consumption of common bean at 40 kg per person (Uganda Bureau of Statistics 2010; Broughton et al., 2003). Interest exists in biofortified crops in Uganda due to the heavy reliance on staples for vitamins and minerals, sweet potatoes have been biofortified with high levels of vitamin A and the biofortified varieties are now being grown and consumed in the communities (Hotz et al., 2012). Biofortified beans developed based on seed iron concentration have been developed and are being released by HarvestPlus in Uganda. The HarvestPlus genotypes include two yellows (MOORE 88002 and Nyiramuhondo), one red mottled (RWR 2245), and one cranberry (RWR 2154). Variety Nyiramuhondo is an indeterminate climber while the other three genotypes are all determinate (bush) types (http://www.icrisat.org/release-of-biofortified-bean-varieties-in-uganda/).

To be adopted by the end users, biofortified crops must be high yielding, profitable, efficacious, and effective at reducing micronutrient deficiency in humans, as well as acceptable to farmers and consumers (Hortz et al 2007). Participatory variety evaluation and selection combines the views of farmers and researchers and is thus well suited for variety evaluation and development for the smallholder farmers (Gyawali et al 2007). Evaluation of these genotypes with famers in the different agro-ecological zones of Uganda will give us knowledge about their performance in an environment where they could potentially be released as varieties or used in the national program for improvement of existing varieties for mineral concentration and bioavailability. Plant breeders have largely focused on development of varieties for improved agronomic traits like yield and disease resistance with less effort targeted on nutritional quality and consumer traits (Luby and Shaw, 2009). With the increased interest in nutritional breeding and biofortification, it is also vital to look at sensory characteristics of biofortified varieties. Limited adoption of new crop varieties occurs in developing countries. For instance, the presence of mismatches between dry bean breeders' goals vs. the needs, expectations, and preferences of the growers and consumers. Ideally, growers demand common bean cultivars pyramided with multiple traits for their multiple needs and any variety that falls short in one trait is likely to be rejected (Belon, 2002). Additionally, absence of expected levels for subjective attributes like flavor, texture, cooking quality, and seed color can turnoff growers and consumers (Almekinders and Elings, 2001; Gyawali et al., 2007).

Human sensory evaluation of foods is a powerful methodology for estimation of how consumers will react to a new food product or variety. The method enables consumers to provide information about food properties and the interpretation of such properties (Lawless and Heyman, 2010; Meilgaard et al., 2007). Sensory evaluation of foods can be broadly divided into two groups i.e. objective (analytical) measurements (where trained assessors are used) and subjective

(affective) where consumers are used to give their opinions about the product or variety (Meilgaard et al., 2007). With objective measurements, assessors (panelists) are usually few (30 to 60) and have to be intensively screened, trained, and monitored to look at their performance in evaluating a product or variety (Meilgaard et al., 2007). Training sessions for analytical sensory panels can last between 30 to 60 days and therefore this method is slow and expensive. With subjective (consumer affective) sensory measurements, the panelists (consumers) require minimal training as long as they have prior experience with using the food product and therefore large numbers of panelists from 100 to greater than 1,000 can be recruited to provide hedonic judgments about the sensorial properties of the new product or variety (Lawless and Heyman, 2010). Information collected during consumer hedonic tests include what consumers like about the new product, how much they like it, intent to purchase and/ or grow the new variety, and their overall preference or acceptance of the new product (Meilgaard et al., 2007).

The subjective consumer sensory evaluation can be conducted in a laboratory setting, at as a central location trial (CLT) also called Hall test (HT), and as a Home Use Trial (HUT) (Lawless and Heyman, 2010). Since one of the major goals of consumer affective sensory test is to elicit natural behavior from the panelists about the product a laboratory test tends to be artificial. With the CLT, an appropriate central location such as a school, community hall, canteen, or church can be identified and consumers brought to that facility to complete the sensory evaluation of the new product (Lawless and Heyman, 2010). Researchers are also present to offer clarifications or explain rating instructions. With the HUT methodology, the researcher sends the new product to the panelists' homes so that they can try it in their normal eating environment and then give feedback. While HUT is the most natural method for the consumers to evaluate a product and cheaper to the researchers, it can have challenges. For instance, concerns about compliance

(whether people do exactly what they have been asked to do) and filling in questionnaires correctly especially when some respondents can't read or write (Meilgaard et al., 2007).

The factors that can affect consumer sensory evaluations and food choices include demographic characteristics (age, gender, household size), education, economic assets (e.g. land), social capital, presence or lack of alternative crops (Vabo and Hansen, 2014). Other factors include the food itself based on its physical and chemical characteristics (Shepherd, 1999). The physical and chemical composition (proteins, carbohydrates, minerals, and bioactive compounds) will influence sensorial attributes like flavor, texture, taste, and appearance (Cardello, 1994; Mkanda et al., 2007).

Plant breeders now acknowledge that new varieties need to be evaluated for consumer quality traits in addition to agronomic performance as has been in the past. As a consequence, assessment of consumer sensory characteristics is a growing component of variety evaluation and adoption studies in Africa. For instance, consumer sensory evaluation has been used for new varieties of vegetable pea in Kenya (Ojwang et al., 2016), pro-vitamin A biofortified maize in Mozambique, South Africa, and Zimbabwe (Stevens and Winter-Nelson, 2008; Pillay et al, 2011; Muzinghi et al., 2008), biofortified cassava in Kenya (Talsma et al., 2013), and sweet potatoes in Tanzania and South Africa (Tomlins et al., 2007; Laurie et al., 2012). There is limited consumer sensory evaluation data on nutrient dense common beans. The objectives of this study were two-fold: First, to determine farmers' priority traits present in biofortified common bean genotypes. Second, to identify common bean genotype differences with respect to their sensory attributes of flavor, texture, and taste.

Materials and Methods

Farmer group selection and collection of socio-economic data

Nine farmer groups were visited in November, 2014 to introduce the research project on high mineral and fast cooking beans. The farmer groups were from the districts of Hoima (western Uganda), Kamuli (eastern Uganda), Masaka, and Rakai districts (both in central Uganda) in Uganda. The farmer group members were comprised of both male and female members with each group having about 30 members (or 270 in total). The female members were 195 while the males were 75. The farmers were informed about the project and consulted about their interest to participate in the research through consent based on Michigan State University's Institutional Review Board approval for this research work.

For a group to be selected, farmer group members had to have an interest in bean production and consumption. From the 270 members we selected only 153 participants and interviewed them about their socio-economic characteristics. Information relevant to bean production like bean acreage farmed, bean marketing, seed types grown, and alternative crops grown was collected during the socio-economic survey sessions. Bean consumption related information such as how often household members consume beans, what seed types are consumed, and bean cooking times were also collected.

Plant materials and farmer selection of top four genotypes

The dry bean genotypes evaluated came from 15 test entries plus a local check grown at nine locations for the larger genotype by environment experiment described in Chapter 2. The farmers were asked to identify the four best genotypes at harvest and describe reasons by giving both positive and negative traits about the preferred genotypes at harvest (Table 3.6). Once the four best genotypes were identified, accessions were then packaged to collect agronomic data and remove samples for nutritional quality and cooking time assessments. The selected four best

genotypes plus the local check from the nine locations were then bulked to have enough seed for all 108 panelists to evaluate the sensory attributes at the three central locations.

Selection of consumer sensory panelists

A total of 108 panelists were recruited to participate in the consumer sensory evaluations. All panelists were selected based on following criteria: (a) their availability to participate in the sensory evaluation, (b) being healthy and able to participate in the research, (c) had no known allergies or intolerances to dry beans, (d) were interested and willing to participate in the bean sensory evaluations, (e) had ability to discriminate and describe sensory attributes of food, and (f) showed sensory acuity for taste, smell, and vision. A total of 108 panelists that met the above criteria were identified to evaluate the five common bean genotypes (Ervilha, PI527538, Rozi Koko, Chijar, and NABE-15).

The cultivation status and sources of origin of the sensory genotypes is as follows: Ervilha is a yellow landrace from Angola in Southern Africa; PI527538 is a yellow bean landrace from Burundi in East Africa; Rozi Koko is a red mottled landrace from Tanzania in East Africa; Chijar is a red mottled landrace from the Caribbean; and NABE-15 (Kanyeebwa) is a popular local check variety released by National Crops Resources Research Institute in 2008. NABE-15 is cream mottled, early maturing, high yielding, and resistant to anthracnose, common bean rust, and halo blight, and fast cooking. We limited our bean sensory servings to a maximum of five samples (genotypes) because past literature suggests that with ratings of more than five samples panelists start getting adaptation effects (i.e. change in behavior from the previous sample to the next sample) which can in turn be confusing to them (Meilgaard et al., 2007).

Preparation of the bean samples for sensory evaluation

The beans were prepared out of sight of the sensory panelists where six experienced bean chefs that did not participate in the sensory evaluation were tasked with preparing the beans and ensuring they were fully cooked and had adequate salt. The procedure for cooking the dry beans was as described in del Castillo et al., (2012) with some minor modifications. Briefly, beans were sorted to remove any malformed grains, washed, and then placed in a 9-liter size stainless steel pot filled with clean cooking water. Bean cooking was conducted using firewood for all the five genotypes at the three central locations. During cooking the pan was always covered with a lid and water level above the beans maintained at 1cm by refilling water as necessary. Occasional opening of the pot during bean cooking was done by the chefs to allow the steam to escape.

Salt was added when the beans were cooked at a ratio of approximately 5 g of salt to 500 g of dry beans. Apart from salt, no additional ingredients were added to the bean samples prior to sensory evaluations by the panelists. The beans were considered cooked when they were soft enough to be eaten and all the six experienced chefs had reached an agreement through successive sampling and tasting. Beans were allowed to cool and then served to all of the 108 consumer panelists for sensory evaluation. Bean cooking at all the three central locations started around 10:00 am local time and consumer sensory evaluations started between 12:00 to 1:00 pm and continued until all panelists were served.

Consumer sensory evaluations

The consumer sensory evaluation was only conducted in 2016 because seed harvests from 2015 had to be saved for the second season's planting in March 2016. For the sensory evaluation we used the central location trial approach where all the 36 panelists from each district converged at one of the centrally located farmer group's community hall to participate in the bean evaluation.

Panelists were served with 50 g of cooked beans placed in a disposable plastic bowl of each of the five genotypes. All panelists assessed each genotype once and were provided with bottled mineral water and crackers to use as palate cleansers between samples/genotypes. A structured 9-point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; and 1 = dislike extremely) was used for the evaluation of bean and broth color, overall appearance, flavor, taste, and overall acceptance. For genotype texture we used a 5-point hedonic scale (5 = much too firm; 4 = a little too firm; 3 = just about right; 2 = a little too soft; 1 = much too soft).

Statistical data analyses

All sensory data were subjected to analysis of variance (ANOVA) using PROC GLM in SAS and correlations among sensory attributes were determined using the PROC COR statement also in SAS v9.4 (SAS-Institute-Inc 2011). Descriptive summary statistics for socio-economic data were also generated. Genotypic mean separation for all common bean sensorial attributes was completed using the Tukey multiple pair-wise comparison test at a significance *P* value of 0.05 in SAS. To determine regional preferences for the study genotypes, sensory data from the three locations was analyzed separately using the statistical model shown below:

$Y = G + P + G \times P + \varepsilon$; where Y is the dependent variable like flavor, taste, or texture; G is effect of one of the six common bean genotypes; P is the effect of the consumer sensory panelists; G x P denotes the interaction between common bean genotypes and consumer panelists; while ε is the error term.

Results and Discussion

Socio-economic characteristics of the study respondents

The socio-economic data collected, indicated that the majority respondents were women at 75.2 % and the average age of all respondents was 41.8 years (Table 3.1). This underscores the enormous role of women in the agriculture workforce as numerous studies have also reported more women being involved in farming especially in developing countries (Baanante et al., 1999; Buvinic and Gupta, 1997; de Haen et al., 2003; Ellis et al., 2006). Having more women involved in agriculture is also useful especially for nutrient dense crops since they are usually the primary care givers for the major target group (children under five years) of nutritional programs (Das et al., 2013). The average land acreage allocated to beans was 0.33 ha which is typical size for small holder farmers. Bean consumption was also high with most respondents mentioning that they consume beans about five times a week on average (Table 3.1). Uganda is one of the countries with the highest per-capita bean consumption in sub-Saharan Africa (Akibode and Maredia, 2012; Uganda Bureau of Statistics 2010). About 87 % of the respondents mentioned they are involved in some form of dry bean marketing either to the local traders or school lunch programs. Fuel wood was the main source of energy for cooking beans in the rural communities with firewood (94.8 %) and charcoal (15.7 %). A small percentage of respondents (14.4 %) mentioned use of soaking beans before cooking to reduce the long cooking times. Cooking times for dry beans averaged at 117.6 minutes using fuel wood as the source of cooking energy (Table 3.1). This illustrates the need to develop fast cooking beans for these communities.

Bean production and consumption

On the choice of seed types grown and consumed, it was clear that most growers and consumers like red mottled dry beans as 98, 90.2, and 96.1 of the respondents identified themselves as growers of this market class in Hoima, Kamuli, and Masaka districts respectively (Table 3.2). Yellow beans were second in preference for Hoima and Kamuli district at 84.3 and 78.4 % respectively (Table 3.2). White beans were the second preferred market class for production in Rakai district at 60.8 % followed by yellow beans at 45.1 % (Table 3.2). Cranberry was mostly grown in Hoima (51 %) followed by Kamuli (41.2 %). Only respondents from Hoima (7.8 %) mentioned production of purple speckled beans while Kamuli and Rakai districts had no respondents for this seed type making it the least popular (Table 3.2). Seed typed for consumption also followed the production trends where red mottled beans were the most consumed across the three districts with 98, 96.1, and 94.1 % for Hoima, Kamuli, and Rakai districts respectively (Table 3.3). Yellow beans were the second most widely consumed beans in Hoima and Kamuli districts while white beans were the second most preferred in Rakai. More respondents consumed cranberry beans in Hoima (58.8 %) than in Kamuli (37.3 %) and Rakai (41.2 %) respectively (Table 3.3). Our results are similar to previous studies that showed that red mottled beans are one of the most preferred seed type by growers and consumers in Eastern Africa (Katungi et al., 2009). It was interesting to note that some respondents from all the three districts grow and consume variety mixtures (composites) (Table 3.2 and 3.3). Cultivar mixtures or multilines refer to different varieties with uniform morphological traits but with varying levels of insect and disease resistance that are grown together in a single plot/ field to ensure yield stability and reduce chemical pesticide use (Browning and Frey, 1981; Mundt, 2002). In Uganda, common bean multilines have been deployed to minimize the damage caused by bean flies (*Ophiomyia* spp.) in farmers' fields (Ssekandi et al., 2016).

We were also interested in understanding the reasons/ motivations for why respondents grow or consume certain seed types (Table 3.4 and 3.5). Over 25 % of the respondents from Hoima, Kamuli, and Rakai districts mentioned that their choice for seed types to grow or consume is based on traditions or customs (Table 3.4 and 3.5). If a given market class is widely grown and consumed in a region or family, children tend to continue that tradition of producing and consuming the market class when they grow up (Chowdhury et al., 2011; Sambodo and Nuthall, 2010). Over 90 % of the respondents in Hoima and Kamuli districts reported growing bean varieties based on the marketability, earliness to maturity, and seed yield (Table 3.4). In Rakai district, over 80 % of the respondents mentioned that their choice for bean varieties to grow is based on seed availability, earliness to maturity, yield, and availability (Table 3.4). Varieties/ seed types that mature early are going to be adopted by the growers as they fetch premium prices in the market. Cooking time was also important to the respondents across the three districts with 74.5 % in Hoima, 82.4 % in Kamuli, and 92.2 % in Rakai mentioning it as a priority trait in choosing bean genotypes to grow (Table 3.4). Seed color was important to growers in Hoima (62.7 %), Kamuli (56.9 %), and Rakai (70.6 %) districts (Table 3.4). Drought tolerance and pest and disease tolerance traits were mostly important to growers in Hoima districts at 78.4 and 76.5 % respectively (Table 3.4). In both Kamuli and Rakai districts 41.2 % of the respondents mentioned drought as a major factor in bean variety selection for production. Drought tolerant bean varieties are particularly important given the weather and climate changes recently reported in our study districts (Mubiru et al., 2015). Pest and disease resistance was at 31.4 % and 25.5 % for respondents in Kamuli and Rakai districts (Table 3.4).

Over 90 % of the respondents in the districts of Hoima and Kamuli reported cooking time and flavor as the drivers of what they end up consuming (Table 3.5). In Rakai district, 90.2 % of

the respondents mentioned that they valued bean flavor while 84 % considered cooking time as a priority for beans to consume (Table 3.5). This makes cooking time and bean flavor important consumer traits in the study areas. Bean availability at 82.4 % was an important factor for people in Hoima district while over 95 % of the respondents in both Kamuli and Rakai districts considered availability as an important factor (Table 3.5). The fact that such a high percentage of farmers are willing to plant or consume bean varieties based on what is available not what can give them an advantage in the market or stronger benefit to their nutrition and health status is a testament of the weak seed and agribusiness systems present in many countries in sub-Saharan Africa (Otieno et al., 2017). Over 70 % of the respondents in Hoima and Kamuli districts valued bean seed size and color as factors for bean variety selection while 68.6 % valued seed color in Rakai district. In Hoima district, 76.5 % of the respondents mentioned health and nutrition of bean varieties as important while 70.6 and 58.8 % of the respondents in Kamuli and Rakai districts valued this trait. The fact that over 50 % of all respondents considered nutrition and health provided by the beans, suggests that they are now aware of the nutritional benefits of beans like provision of iron and zinc for their general health and wellbeing. Bean texture was an important consumer trait for 62.7 % of the respondents in Hoima, 41.2 % in Kamuli, and 54.9 % in Rakai districts (Table 3.5).

Farmers' characteristics of the studied common bean genotypes

The particular crop characteristics that growers paid close attention to during plant growth, harvesting, and processing of the five genotypes are presented in Table 3.6. Among the positive traits, early maturity, plant growth habit and yield were dominant themes for good genotypes as growers preferred upright architecture and high yield. Seed size and color were also important especially large seeded beans with red mottled and yellow market classes. The growers preferred early maturing genotypes, disease resistant, with long pods, and clean canopy (shades off leaves

at maturity) (Table 3.6). The anticipated bean broth color and market potential based on seed color and yield were also favorable traits considered by growers (Table 3.6). Negative traits included susceptibility to disease pressure, few and short pods, small seed size, inadaptability to changing weather patterns and less fertile (poor) soils (Table 3.6). It was interesting to note that if a variety had some negative traits like viny growth habit, late maturity, and small seed size but was high yielding and had the preferred seed color (especially red mottled) then growers would compromise and select it like they did for Chijar (Table 3.6). Overall, the farmers' priority traits and preferred seed types mirrored the results from the survey data we collected at the beginning of the research work in the 2015 field season (Tables 3.4 and 3.5).

Bean cooking time at the sensory evaluation locations

With fuelwood as source of energy, the cooking times for the five bean genotypes ranged from 102 to 124 minutes for Hoima district with genotype PI527538 cooking for the longest time while NABE-15 (local check) and Ervilha took the shortest time to cook (Table 3.7). In Kamuli district, cooking time ranged from 105 to 127 minutes with NABE-15 taking the shortest time to cook. In Rakai district, cooking time varied from 100 to 125 minutes with Ervilha taking the shortest time (Table 3.7).

Genotype effects on sensory attributes at the three central locations

The ANOVA showed significant effect of genotypes on bean sensory attributes evaluated (*P* value = 0.001) by the panelists at the three central locations of Hoima, Kamuli, and Rakai (Tables 3.8 to 3.10). In Hoima district, the cream mottled local check (NABE-15) had a higher bean and broth color rating compared to landraces Rozi Koko and PI527538 (Table 3.8). NABE-

15 and the yellow bean landrace Ervilha had higher appearance scores compared to the red mottled landrace Rozi Koko. Genotypes Ervilha and NABE-15 were more flavorful to the sensory panelists compared landraces Rozi Koko and PI527538 (Table 3.8). The local check NABE-15 tasted better to the Hoima district sensory panelists than Rozi Koko and PI527538. Genotypes Ervilha and NABE-15 were softer compared to Rozi Koko, Chijar, and PI527538 based on seed texture ratings. These two accessions were also fast cooking and the Hoima district panelists found them to be softer during the biting and chewing process of the sensory evaluation (Tables 3.7 and 3.8). Genotypes Ervilha and NABE-15 were the most acceptable to the sensory panelists compared to Rozi Koko and PI527538 at the Hoima central location.

At the Kamuli central location, Rozi Koko had a better bean and broth color compared to the local check NABE-15 and PI527538 (Table 3.9). Genotypes Rozi Koko and the yellow bean Ervilha received higher appearance scores than NABE-15 and PI527538 in Kamuli (Table 3.9). Genotypes Ervilha and Rozi Koko were the most flavorful to the Kamuli sensory panelists when compared to NABE-15 and PI527538 (Table 3.9). The yellow bean Ervilha and red mottled Chijar and Rozi Koko tasted better to the Kamuli panelists compared to NABE-15 and PI527538. The genotype PI527538 had the highest seed texture score while Ervilha had the lowest texture rating. These two genotypes had opposite cooking times in Kamuli with Ervilha cooking fastest while PI527538 taking longer time to cook (Table 3.7). Genotypes Ervilha and Rozi Koko had high acceptance scores to the Kamuli panelists compared to NABE-15 and PI527538 (Table 3.9).

In Rakai district, Rozi Koko, NABE-15, and Chijar, had higher bean and broth scores compared to PI527538, and a similar pattern was observed for the overall bean appearance ratings at this location (Table 3.10). There were no significant differences among the common bean

genotypes for flavor, taste, and overall acceptance (Table 3.10). Seed texture scores were lowest in Ervilha and NABE-15 compared to the red mottled landrace Chijar (Table 3.10).

Genotype Ervilha had consistently low texture scores across the three locations and high overall acceptance in Hoima and Kamuli districts. Seed texture is an important sensorial trait to bean consumers (Sanzi and Atienza, 1999). The bean consumers mostly preferred softer bean genotypes like Ervilha and NABE-15 compared to the genotype PI527538 with high texture scores in Hoima and Kamuli districts (Tables 3.8 and 3.9). These results are consistent with the findings of Ghasemlou et al., (2013) working on cooked beans, they reported that the highest rejection and presence of undesirable sensory attributes was observed among genotypes with the highest firmness (texture) values. Indeed, in our study, the landrace PI527538 had high texture scores and was among the least acceptable genotypes to the sensory panelists.

Additional crops grown by the surveyed farmers

Since beans are grown and consumed along with other staples in a complementary manner, we were interested to determine which crops are widely grown and consumed along with beans in the study locations. Over 90 % of the growers in Hoima, Kamuli, and Rakai districts reported that they grow cassava, maize, and sweet potatoes (Table 3.11). Ground nuts at over 84 % in all three districts was the next widely grown legume crop. Irish potato was an averagely grown staple in Hoima (43.1 %) and 33.3 % in Rakai with very low production in Kamuli district (Table 3.11). Cooking banana was a highly grown in Kamuli (80.4 %) and Rakai (70.6 %) districts followed by Hoima (64.7 %). Leafy vegetables were widely grown in Hoima and Rakai with both districts reporting 76.5 % while in Kamuli 56.9 % of the respondents reported production of leafy vegetables. Millet is a widely grown staple in Hoima district at 52.9 % with less production of this

crop in Rakai (11.8 %) district (Table 3.11). Rice was the least produced crop as only 15.7, 17.6, and 1.9 % of our respondents answered "yes" to growing it (Table 3.11).

There is growing interest in *in vivo* feeding and efficacy trials for biofortified dry beans to determine how effective biofortified beans are effective in combatting Fe malnutrition (Tako et al., 2016; Reed et al, 2017; Haas et al., 2016; Murray-Kolb et al., 2017). This information on complimentary crops grown and consumed can be useful in designing diets for feeding trials in poultry models as well human studies to further ascertain the efficacy of the biofortified dry beans identified in our studies in combatting Fe malnutrition in the future.

Conclusion

Common bean is an important crop to both growers and consumers in Uganda. Our survey results showed that people consume beans up-to 5 days a week making it an important food security and cash crop. Our results also showed that nutritional composition and consumer traits are important to growers as are agronomic traits. The existing Ugandan bean varieties can certainly be improved for traits like yield, mineral composition, cooking time, flavor, and taste so that they can effectively meet the needs of the growers and consumers alike. This study helped provide information on farmer's priorities with regards to variety selection and demonstrated the usefulness of involving farmers and consumers in the evaluation of bean germplasm as part of the variety development, evaluation, and selection process. The knowledge generated in this study will be useful in parental selection for breeding to further improve common bean but also in the design of feeding trials to determine efficacy of biofortified beans.

Table 3.1: Socio-economic characteristics of bean growers in Hoima, Kamuli, and Rakai districts of Uganda

Variable	Response (N=153) Mean (SD)
% of female respondents	75.2
Age of the respondents	41.8 (12.1)
Bean acreage usually farmed (ha)	0.33 (0.34)
Number of days the household consumes beans in a week	5.1 (1.6)
% of respondents who soak beans before cooking	14.4
% of respondents who sell some of their bean produce	87.6
% of respondents who use firewood for cooking	94.8
% of respondents who use charcoal for cooking	15.7
Number of minutes it takes for the beans to cook	117.6 (42.1)

SD: standard deviation of the mean

Table 3.2: Farmers' choice of bean seed types for production in Hoima, Kamuli, and Rakai districts in Uganda

	% of respondents growing seed type		
Bean seed type	Hoima	Kamuli	Rakai
Red mottled	98	90.2	96.1
Yellow	84.3	78.4	45.1
White	62.7	68.6	60.8
Cranberry	51	41.2	25.5
Purple speckled	7.8	-	-
Composite (variety mixtures)	31.4	7.8	9.8

Table 3.3: Farmers' choice of bean seed types for consumption in Hoima, Kamuli, and Rakai districts in Uganda

	% of respondents consuming seed type		
Bean seed type	Hoima	Kamuli	Rakai
Red mottled	98	96.1	94.1
Yellow	84.3	60.8	70.6
White	76.5	52.9	72.5
Cranberry	58.8	37.3	41.2
Purple speckled	37.3	-	-
Composite (variety mixtures)	58.8	9.8	3.9

Table 3.4: Reasons for farmers' choice of common bean seed types to grow in Hoima, Kamuli, and Rakai districts in Uganda

	% of "yes" as the reason to grow a particular seed type		
Trait / reason	Hoima	Kamuli	Rakai
Tradition or custom	29.4	25.5	35.3
Cooking time	74.5	82.4	92.2
Seed size and color	62.7	56.9	70.6
Health and Nutrition	78.4	72.5	58.8
Availability	68.6	76.5	84.3
Price and Marketability	94.1	96.1	90.2
Drought tolerance	78.4	41.2	41.2
Early maturity	92.2	96.1	94.1
Pest and disease resistance	76.5	31.4	25.5
Seed yield	94.1	100	84.3

Table 3.5: Reasons for farmers' choice of common bean seed types to consume in Hoima, Kamuli, and Rakai districts in Uganda

	% of "yes" as the reason to consume a particular seed type		
Trait / reason	Hoima	Kamuli	Rakai
Tradition or custom	31.4	39.2	27.5
Texture	62.7	41.2	54.9
Flavor	90.2	92.2	90.2
Cooking time	94.1	92.2	84.3
Seed size and color	78.4	72.5	68.6
Health and Nutrition	76.5	70.6	58.8
Availability	82.4	96.1	98
Price and Marketability	64.7	21.6	11.8

Table 3.6: Bean genotype characteristics emphasized by growers during flowering, harvesting, and at threshing as their top five choices in Uganda

Genotype	Positive traits	Negative traits
Ervilha	Upright plant architecture, has long pods, early maturity, uniform maturity, clean canopy (shades off leaves at maturity), large seed size, good seed color, good yields, has market potential	Has high disease pressure, suffers from change in weather patterns and poor soils
Rozi Koko	Upright plant architecture, low disease pressure, early maturity, uniform maturity, clean canopy (shades off leaves at maturity), has long pods, large seed size, good seed color, likely to have good broth color upon cooking, has market potential	Has few pods, low yields, suffers from change in weather patterns and poor soils
Chijar	Low disease pressure for most diseases, adaptable to changing weather patterns, heavy pod load (has numerous pods), very high yielding, good seed color, likely to have good broth color upon cooking, has market potential	Has viny/ climbing growth habit, many plants wilted due to black root hypersensitive response, late maturity, small seed size
PI527538	Upright plant architecture, early maturity, clean canopy (shades off leaves at maturity), heavy pod load, large seed size, good seed color, high yielding	Has high disease pressure, hard seed coat, slow cooking
NABE-15	Upright plant architecture, high yielding, makes good broth from past experience with the variety, fast cooking	Has high disease pressure, non-uniform maturity, suffers from change in weather patterns and poor soils

Table 3.7: Cooking time of the five genotypes evaluated in the sensory study at the three central locations in Hoima, Kamuli, and Rakai districts of Uganda

	Cooking time (minutes)		
	Location		
Genotype	Hoima	Kamuli	Rakai
Rozi Koko	109	120	107
NABE-15	102	105	101
Chijar	111	117	120
Ervilha	105	114	100
PI527538	124	127	125

Table 3.8: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Hoima district of Uganda

	Sensory attribute					
Genotype	Bean and broth color	Appearance	Flavor	Taste	Texture	Overall acceptance
Rozi Koko	6.7 b	6.7 c	6.6 c	6.5 c	3.1 a	6.4 c
NABE-15	7.6 a	7.7 a	7.6 a	7.8 a	2.1 b	7.8 a
Chijar	7.2 ab	7.1 abc	7.4 ab	7.5 ab	2.8 a	7.2 ab
Ervilha	7.3 ab	7.5 ab	7.8 a	7.7 ab	1.9 b	7.9 a
PI527538	6.8 b	6.9 bc	7.1 bc	7.2 b	2.8 a	7.0 bc

Genotype means followed by the same letter within each column are not different according Tukey multiple pair-wise comparison test ($P < 0.05$)

Table 3.9: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Kamuli district of Uganda

	Sensory attribute					
Genotype	Bean and broth color	Appearance	Flavor	Taste	Texture	Overall acceptance
Rozi Koko	7.9 a	7.7 a	7.8 a	7.7 a	2.6 b	7.7 a
NABE-15	6.7 bc	6.5 b	6.4 bc	6.5 b	2.4 b	6.7 bc
Chijar	7.2 ab	7.2 ab	7.3 ab	7.6 a	2.6 b	7.4 ab
Ervilha	7.3 ab	7.6 a	7.7 a	8.0 a	1.7 c	7.9 a
PI527538	6.1 c	6.4 b	6.1 c	6.3 b	3.2 a	6.4 c

Genotype means followed by the same letter within each column are not different according Tukey multiple pair-wise comparison test ($P < 0.05$)

Table 3.10: Genotype effects on the evaluated sensory attributes by 36 consumer sensory panelists in Rakai district of Uganda

	Sensory attribute					
Genotype	Bean and broth color	Appearance	Flavor	Taste	Texture	Overall acceptance
Rozi Koko	7.1 a	6.7 a	6.7 a	7.1 a	3.1 ab	6.6 a
NABE-15	7.2 a	7.1 a	6.6 a	7.0 a	2.6 bc	7.0 a
Chijar	7.0 a	6.8 a	7.0 a	7.2 a	3.4 a	6.8 a
Ervilha	6.6 ab	7.1 a	6.9 a	6.9 a	2.0 c	6.9 a
PI527538	5.8 b	6.2 b	6.7 a	6.9 a	2.9 ab	6.4 a

Genotype means followed by the same letter within each column are not different according Tukey multiple pair-wise comparison test ($P < 0.05$)

Table 3.11: List of other crops grown by the surveyed dry bean farmers in the districts of Hoima, Kamuli, and Rakai in Uganda

	% of respondents growing the crop		
Crop	Hoima	Kamuli	Rakai
Cassava	98	94.1	90.2
Sweet potatoes	94.1	96.1	94.1
Maize	90.2	98	100
Irish potatoes	43.1	7.8	33.3
Ground nuts	86.3	86.3	84.3
Cooking bananas	64.7	80.4	70.6
Millet	52.9	47.1	11.8
Leafy vegetables	76.5	56.9	76.5
Tomatoes	43.1	35.3	27.5
Rice	15.7	17.6	1.9

LITERATURE CITED

LITERATURE CITED

Akibode, C.S., Maredia, M. 2011. Global and regional trends in production, trade and consumption of food legume crops. Report submitted to the Standing Panel on Impact Assessment (SPIA) of the CGIAR Science Council, FAO, Rome.

Almekinders, C.J.M., Elings, A., 2001. Collaboration of farmers and breeders: participatory crop improvement in perspective. Euphytica 122:425-438.

Baanante, C., Thompson T.P., Acheampong, K., 1999. Labour contributions of women to crop production activities in three regions of West Africa: An analysis of farm-survey data. Institute of African Studies: Res. Rev. 15:80-100.

Beebe, S., 2012. Common bean breeding in the tropics. Plant Breed Rev 36:357-426.

Bellon, M.R. 2002. Analysis of the demand for characteristics by wealth and gender: A case study from Oaxaca, Mexico. In Quantitative analysis of data from participatory methods in plant breeding, ed. M.R. Bellon and J. Reeves, 66-81. Mexico: CYMMYT.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J., 2003. Beans (*Phaseolus* spp.)-model food legumes. Plant Soil 252:55-128.

Browning, J.A., Frey, K.J., 1981. The multiline concept in theory and practice. In: Jenkyn, J.F., Plumb, R.T., (eds) Strategies for the control of cereal disease. Blackwell Scientific, London, pp 37-39.

Buvinic, M., Gupta, G.R., 1997. Female-headed households and female-maintained families: Are they worth targeting to reduce poverty in developing countries? Econ. Dev. Cult. Change 45:259-80.

Cardello, A.V., 1994. Consumer expectations and their role in food acceptance. Mac Fie, H.J.H., Thomson, D.M.H., (Eds.). Measurement of food preferences, Blackie Academic & Professional, London p.253.

Chowdhury, S., Meenakshi, J.V., Tomlins, K., Owori, C., 2011. Are consumers in developing countries willing to pay more for micronutrient-dense biofortified foods? Evidence from a field experiment in Uganda. Am. J. Agric. Econ. 93:83-97.

Das, J.K., Salam, R.A., Kumar, R., Bhutta, Z.A. 2013. Micronutrients food fortification and its impact on woman and child health: A systematic review. Syst. Rev 2:1-24.

de Haen, H., Stamoulis, K., Shetty, P., Pingali, P., 2003. The world food economy in the twenty-first century: Challenges for international co-operation. Dev. Policy Rev. 21:683-696.

del Castillo, R.R., Costell, E., Plans, M. Simó, J., Casañas, F., 2012. A standardized method of preparing common beans (*Phaseolus vulgaris* L.) for sensory analysis J. Sens Stud. 27:188-195.

Ellis, A., Manuel, C., Blackden, C.M., 2006. Gender and economic growth in Uganda: Unleashing the power of women. Washington, D.C.: The World Bank.

Ghasemlou, M., Gharibzahedi, S.M.T., Emam-Djomeh, Z., 2013. Relating consumer preferences to textural attributes of cooked beans: Development of an industrial protocol and microstructural observations. LWT Food Sci. Technol. 50:88-98.

Gyawali, S., Sunwar, S., Subedi, M., Tripathi, M., Joshi, K.D., and Witcombe, J.R., 2007. Collaborative breeding with farmers can be effective. Field Crops Res. 101:88-95.

Haas, J.D., Luna, S.V., Lung'aho, M.G., Wenger, M.J., Murray-Kolb, L.E., Beebe, S., Gahutu, J.B., Egli, I.M., 2016. Consuming iron biofortified beans increases iron status in Rwandan women after 128 days in a randomized controlled feeding trial. J. Nutr. 146:1586-1592.

Hotz C., Loechl, C., Lubowa, A., Tumwiine, J.K., Ndeezi, G., Masawi, A.N., Baingana, R., A. Carriquiry, R., de Brauw, A., 2012. Introduction of β-carotene-rich orange fleshed sweet potato in rural Uganda resulted increased vitamin A intake among children and women and improved vitamin A status among children. J. Nutr. 142:1871-1880.

Katungi, E., Farrow, A., Chianu, J., Sperling, L., Beebe, S., 2009. Common bean in Eastern and Southern Africa: A situation and outlook analysis. International Centre for Tropical Agriculture, CIAT: 61.

Laurie, S.M., Faber, M., Calitz, F.J., Moelich, E.I., Muller, N., Labuschagne, M.T., 2012. The use of sensory attributes, sugar content, instrumental data and consumer acceptability in selection of sweet potato varieties. J. Sci. Food Agric. 93:1610-1619.

Lawless, H. T., Heymann, H. 2010. Sensory evaluation of food: principles and practices (2nd ed.). New York, NY, USA: Springer.

Luby, J.J., Shaw, D.V., 2009. Plant breeders' perspectives on improving yield and quality traits in horticultural food crops. HortScience, 44:20-22.

Mellgaard, M. C., Civille, G. V., and Cart, B. T. 2007. Sensory evaluation techniques (4th ed.). Boca Raton, Florida, USA: CRC Press.

Mkanda, A.V., Minnaar, A., de Kock, H.L., 2007. Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris* L.). Biotechnol. Agron. Soc. Env. 3:201-204.

Mubiru, D.N., Kyazze, F.B., Radeny, M., Zziwa, A., Lwasa, J., Kinyangi, J., 2015. Climatic trends, risk perceptions, and coping strategies of smallholder farmers in rural Uganda. Working paper, CGIAR Research Program on Climate Change, Agriculture and Food Security. 23 June 2015. Climate Change, Agric. and Food Sci., Copenhagen, Denmark. www.ccafs.cgiar.org. (Accessed on 1st November 2017).

Mundt, C.C., 2002. Use of multiline cultivars and cultivar mixtures for disease management. Annu. Rev. Phytopathol. 40:381-410.

Murray-Kolb, L.E., Wenger, M.J., Scott, S.P., Rhoten, S.E., Lung'aho, M.G., Haas, J.D., 2017. Consumption of iron-biofortified beans positively affects cognitive performance in 18- to 27-year-old Rwandan female college students in an 18-week randomized controlled efficacy trial. J. Nutr. 147:2109-2117.

Muzinghi, T., Langyintuo, A.S., Malaba, L.C., Banziger, M., 2008. Consumer acceptability of yellow maize products in Zimbabwe. Food Policy 33, 352-361.

Ojwang, D.J., Nyankanga, R.O., Imungi, J., Olanya, O.M., Ukuku, D.O. 2016. Cultivar preference and sensory evaluation of vegetable pigeon pea (*Cajanus cajan*) in Eastern Kenya. Food Security 8:757-767.

Otieno, G.A., Reynolds, T.W., Karasapan, A., Noriega, I.L., 2017. Implications of seed policies for on-farm agro-biodiversity in Ethiopia and Uganda. Sust. Agric. Res.6:1-19.

Pillay, K., Derera, J., Siwela, M., Veldman, F.J., 2011. Consumer acceptance of yellow, provitamin A-biofortified maize in KwaZulu-Natal. S. Afr. J. Clin. Nutr. 24 (4), 186–191.

Reed S., Neuman, H., Glahn, R.P., Koren, O., Tako, E., 2017. Characterizing the gut (*Gallus gallus*) microbiota following the consumption of an iron biofortified Rwandan cream seeded carioca (*Phaseolus vulgaris* L.) bean-based diet. PLoS ONE 12: e0182431.

Sambodo, L.A., Nuthall, P.L., 2010. A behavioural approach to understanding semi-subsistence farmers' technology adoption decisions: The case of improved paddy-prawn system in Indonesia. J. Agric. Educ.Ext. 16:111-129.

Sanz, M., Atienza, J., 1999. Sensory analysis of beans (*Phaseolus vulgaris*). Biotechnol. Agron. Soc. Environ. 3:201-204.

SAS Institute., 2011. SAS Version 9.4 SAS Institute Inc., Cary, NC, USA.

Shepherd, R., 1999. Social determinants of food choice. Proc. Nutri. Soc. 58:807-812.

Ssekandi, W., Mulumba, J.W., Colangelo, P., Nankya, R., Fadda, C., Karungi, J., Otim, M., De Santis, P., Jarvis, D.I., 2016. The use of common bean (*Phaseolus vulgaris*) traditional varieties and their mixtures with commercial varieties to manage bean fly (*Ophiomyia* spp.) infestations in Uganda J. Pest. Sci., 89:45-57.

Stevens, R., Winter-Nelson, A., 2008. Consumer acceptance of pro-vitamin A biofortified maize in Maputo, Mozambique. Food Policy 33:341-351.

Tako, E., Bar, H., Glahn, R. P., 2016. The combined application of the Caco-2 cell bioassay coupled with i*n vivo* (*Gallus gallus*) feeding trial represents an effective approach to predicting Fe bioavailability in humans Nutrients 8:1-25.

Talsma, E., Melse-Boonstra, A., de Kok, B.P.H., Mbera, G.N.K., Mwangi, A.M., Brouwer, I.D., 2013. Biofortified cassava with pro-vitamin A is sensory and culturally acceptable for consumption by primary school children in Kenya. PLoS ONE 8: e73433.

Tomlins, K., Ndunguru, G., Stambul, K., Joshua, N., Ngendello, T., Rwiza, E., Amour, R., Ramadhani, B., Kapande, A., Westby, A., 2007. Sensory evaluation and consumer acceptability of pale-fleshed and orange-fleshed sweet potato by school children and mothers with preschool children. J. Sci. Food Agric. 87:2436-2446.

Uganda Bureau of Statistics (2010) Uganda Census of Agriculture 2008/2009. Crop area and production report, vol IV, p 178.

Vabo, M., Hansen, H., 2014. The relationship between food preferences and food choice: a theoretical discussion. Int. J. Bus. Soc. Sci. 5:145-157.

GENERAL CONCLUSIONS AND FUTURE RESEARCH

GENERAL CONCLUSIONS AND FUTURE RESEARCH

Micronutrient malnutrition of iron and zinc is a global public health concern as it afflicts up-to two billion people in the world. Common bean is a widely consumed staple crop in Latin America, Africa, and many countries around the world. The crop is high in minerals, fiber, and protein. With its high levels of seed Fe and Zn concentration, it's an appealing target for biofortification programs. For a biofortification program to be successful, there is need for presence of the right germplasm upon which to conduct selection, presence of markers and candidate genes to deploy in marker assisted selection, to ensure that appropriate materials that can effectively address the growers' and consumers' needs are developed. Genetic studies for iron and zinc accumulation in common bean have relied mostly on bi-parental mapping populations with limited marker-data sets, and superior genotypes have not been evaluated with growers to determine if they are stable in growers' locations and if consumers actually like them.

Three studies were designed to address these limitations in common bean nutrition breeding. The first study (Chapter 1) sought to determine genomic loci underlying nutritional related traits in common bean using a novel genome-wide association study (GWAS). A collection of cooked large-seeded Andean beans were used and large variability for seed protein, Fe, Zn, and Ca concentrations and Fe bioavailability was found based on cooked samples. Genomic regions underlying seed protein, calcium, and zinc concentration along with Fe bioavailability were uncovered using GWAS. The SNP markers generated from this experiment will be useful to accelerate marker assisted selections for seed protein, Fe bioavailability, seed zinc, and calcium concentration in common bean. The germplasm identified with superior phenotypic values can be used in making careful breeding crosses for common bean nutritional quality improvement.

In the second experiment (Chapter 2), a set of 15 nutritionally superior genotypes for fast cooking, Fe bioavailability, and seed Fe and Zn phenotypes identified using the GWAS approach

were for on-farm study. These genotypes were evaluated in farmers' fields to determine their yield performance and stability across nine locations for two years in Uganda. We also assessed genotype by environment interaction for yield, foliar diseases, cooking time, and iron and zinc accumulation. This on-farm research component was useful to gain insights into what farmers think about the genotypes and how the genotypes would hold up against local checks given the soil, weather, and field pathogen pressures. Several genotypes that performed well for traits like yield, cooking time, foliar disease resistance, iron and zinc concentrations were identified. The use of GGE biplot analysis helped in the detection of mega-environments for yield, cooking time, and seed iron and zinc concentrations. Discovery of these mega-environments will be useful in selection of sites to evaluate, test, and select common bean genotypes in future dry bean breeding programs.

Working with growers, we were able to select the five best genotypes for which to evaluate sensorial attributes. In Chapter 3 we identified the priority traits and expectations of growers and consumers in a new bean variety. Using the questionnaire survey methodology and consumer sensory evaluations, major traits included yield, seed color, cooking time, nutrient composition, bean flavor, early maturity, and plant architecture. Using the sensory analysis, we identified genotypes that were acceptable to the consumers and these should be useful in breeding for improved nutritional quality traits of common bean. Overall, the new resources that have been generated from this dissertation research such as candidate genes, SNP markers, superior genotypes, trait associations/ correlations, test location information (mega-environments), and grower / consumer priority traits should be useful during decision making to improve common bean for agronomic, quality, and consumer traits.

Recommendations for future research

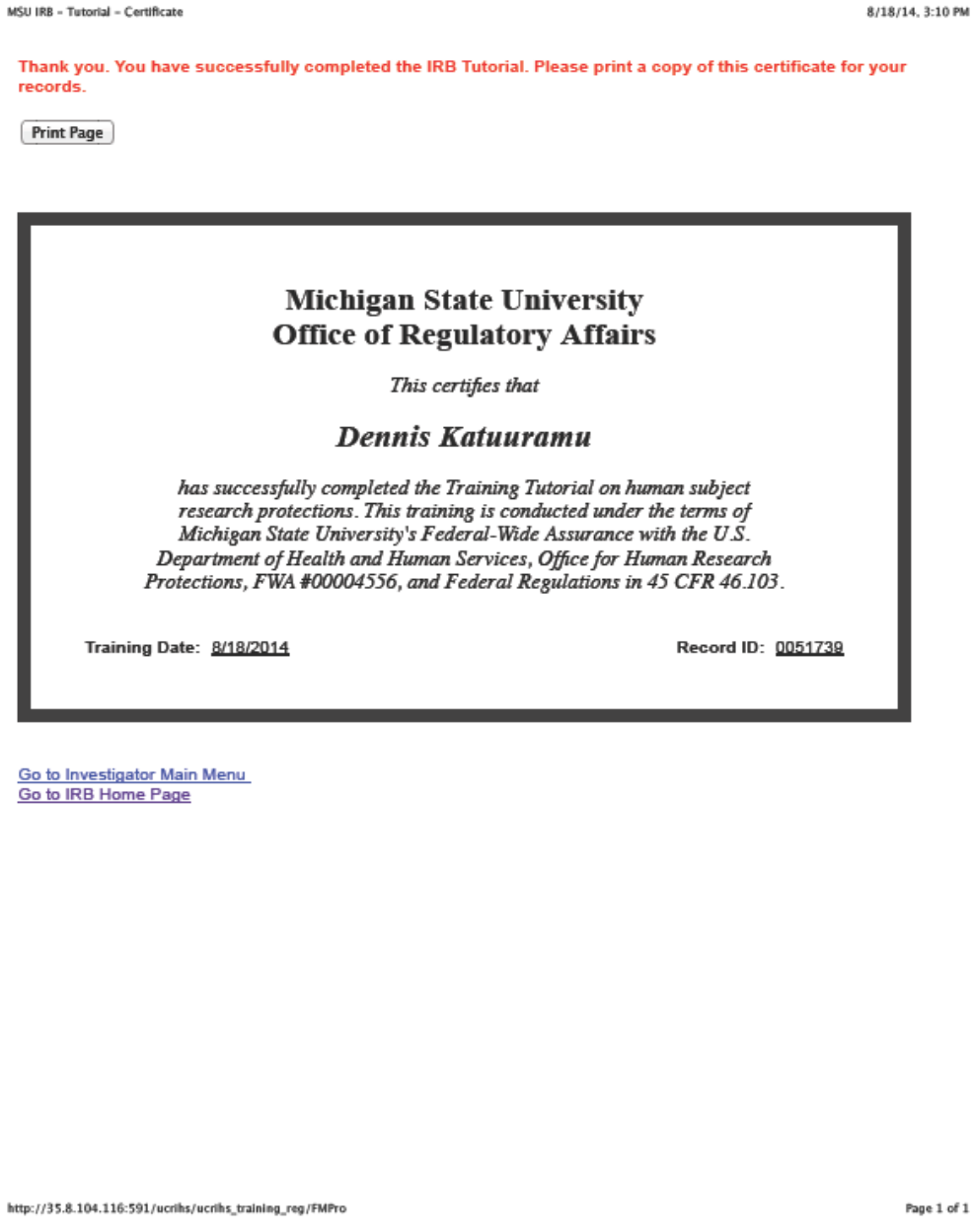
As suggestions for future research, the Andean diversity panel needs to be expanded to at least twice the current size used in the GWAS experiment. It should also be genotyped with more SNP markers preferably those bracketing the linkage disequilibrium (LD) blocks in common bean. Additionally, SNP markers tagging the common bean genes would be more useful as such regions are directly linked to trait control and therefore more informative. Increasing the GWAS panel size will improve the resolution of QTL underlying polygenic traits like seed protein, Fe, and Zn concentration as well as Fe bioavailability. Since most of our exciting accessions for high seed Fe and Zn concentrations, fast cooking, and high Fe bioavailability from the three studies are landraces, these materials should be deployed in crossing blocks to improve the local check varieties for nutritional quality traits. Viable breeding schemes would be backcrossing, recurrent selection, and single seed descent as these allow for most meiotic events to occur.

Size of the household survey and number of consumer sensory panelists should be increased in future studies to reflect opinions of the larger population in these districts. For instance, the study districts of Hoima, Kamuli, and Rakai have a combined population of well over one million people according to the Uganda national household and population census of 2014. Our study sample sizes for both the household survey (153) and consumer sensory panelists (108) are small compared to the actual size of the population in these districts. Furthermore, since a lot of the respondents (over 90 %) in all districts mentioned price and marketability as a key driver of which varieties to grow, future surveys should reflect this finding by interviewing people in both urban and peri-urban communities. This will help gain insights into the potential for commercialization of biofortified and fast cooking beans.

APPENDICES

RESEARCH QUESTIONNAIRES AND IRB DOCUMENTATION

Appendix A: MSU IRB training certificate for Dennis N. Katuuramu



MSU IRB - Tutorial - Certificate 8/18/14, 3:10 PM

Thank you. You have successfully completed the IRB Tutorial. Please print a copy of this certificate for your records.

Print Page

Michigan State University
Office of Regulatory Affairs

This certifies that

Dennis Katuuramu

has successfully completed the Training Tutorial on human subject research protections. This training is conducted under the terms of Michigan State University's Federal-Wide Assurance with the U.S. Department of Health and Human Services, Office for Human Research Protections, FWA #00004556, and Federal Regulations in 45 CFR 46.103.

Training Date: 8/18/2014 **Record ID:** 0051739

Go to Investigator Main Menu
Go to IRB Home Page

http://35.8.104.116:591/ucrihs/ucrihs_training_reg/FMPro Page 1 of 1

Appendix B: MSU IRB approval letter for the research project

MICHIGAN STATE UNIVERSITY

Initial IRB Application Determination *Exempt*

October 2, 2014

To: Karen Cichy
494G PSSB

Re: **IRB# x14-920e** Category: Exempt 6
Approval Date: October 2, 2014

Title: PARTICIPATORY SELECTION AND SENSORY EVALUATION OF BIOFORTIFIED DRY BEAN CULTIVARS IN UGANDA (CGA129499)

The Institutional Review Board has completed their review of your project. I am pleased to advise you that **your project has been deemed as exempt** in accordance with federal regulations.

When you receive the Makerere University IRB approval letter and IRB approved consent form, please forward copy to MSU IRB.

The IRB has found that your research project meets the criteria for exempt status and the criteria for the protection of human subjects in exempt research. **Under our exempt policy the Principal Investigator assumes the responsibilities for the protection of human subjects** in this project as outlined in the assurance letter and exempt educational material. The IRB office has received your signed assurance for exempt research. A copy of this signed agreement is appended for your information and records.

Renewals: Exempt protocols do not need to be renewed. If the project is completed, please submit an ***Application for Permanent Closure***.

Revisions: Exempt protocols do not require revisions. However, if changes are made to a protocol that may no longer meet the exempt criteria, a new initial application will be required.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects and change the category of review, notify the IRB office promptly. Any complaints from participants regarding the risk and benefits of the project must be reported to the IRB.

Follow-up: If your exempt project is not completed and closed after three years, the IRB office will contact you regarding the status of the project and to verify that no changes have occurred that may affect exempt status.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with the IRB office.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at IRB@msu.edu. Thank you for your cooperation.

Sincerely,

H. McGee

Harry McGee, MPH
SIRB Chair

c: Dennis Katuuramu

Office of Regulatory Affairs
Human Research Protection Programs

Biomedical & Health Institutional Review Board (BIRB)

Community Research Institutional Review Board (CRIRB)

Social Science Behavioral/Education Institutional Review Board (SIRB)

Olds Hall
408 West Circle Drive, #207
East Lansing, MI 48824
(517) 355-2180
Fax: (517) 432-4503
Email: irb@msu.edu
www.humanresearch.msu.edu

MSU is an affirmative-action, equal-opportunity employer.

Appendix C: Informed consent form to be completed by the respondents

PROJECT TITLE: PARTICIPATORY SELECTION AND SENSORY EVALUATION OF BIOFORTIFIED DRY BEAN CULTIVARS IN UGANDA

Background information

My name is Dennis Katuuramu, a graduate student at Michigan State University. The purpose of this study is to evaluate the performance of 15 high bioavailable minerals (iron and zinc) beans on farmers' fields in Uganda. Information to be generated will include agronomic traits, cooking time, seed traits, and sensory attributes of the genotypes. Information on existing bean varieties grown by the farmers plus other crops will also be generated.

Participation consent

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time. All farmers belonging to the nine famer groups across the three districts (9x30) or 270 farmers who will grow and evaluate the dry bean varieties will be compensated with a hand hoe. The consumer sensory panelists will be compensated with lunch, notebook, and writing pen.

Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty. You may refuse to answer particular questions. Information from this study will be presented in aggregated form. Your response will not be identified with your name, and your name will be kept confidential.

Do you agree to be interviewed? 1. Yes 2. No

In case you have any concerns or questions about this study, such as scientific issues, report of any form of injury (such as physical, psychological, social, financial, or otherwise) please contact the investigators below.

Dr. Karen A. Cichy
Principal Investigator
Department of Plant, Soil, and Microbial Sciences
Plant and Soil Sciences Building
1066 Bogue St, Room A494G
East Lansing, MI, 48824
Telephone: 517-355-0271 Ext. 1210
Email: cichykar@msu.edu

Dennis N. Katuuramu
Investigator
Department of Plant, Soil, and Microbial Sciences
Plant and Soil Sciences Building
1066 Bogue St, Room A494K
East Lansing, MI, 48824
Telephone: 517-643-0001
Email: katuuram@msu.edu

If you have any questions or concerns about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact anonymously if you wish, Michigan State University's Human Research Protection Program at 517-355-2180, Fax: 517-432-4503 or email irb@msu.edu or via regular mail at 207 Olds Hall, Michigan State University, East Lansing, MI 48824.

Your signature below means that you voluntarily agree to participate in this research study.

Signature: _____ Date _____

Appendix D: Survey instrument for demographic, bean production and consumption characteristics of the respondents in the high mineral dry bean evaluation project in Uganda

DEMOGRAPHIC, BEAN PRODUCTION AND MARKETING QUESTIONNAIRE

Please check the appropriate answer for each of the questions below:

1. Sex _____Male _____Female

2. How old are you? (Age in years) _____

Please answer the following questions. There are no right or wrong answers. We want to know about you and what you think. Please ask if you have any questions!

3. How often do you consume beans?
_____ I do not consume beans
_____ Occasionally
_____ At least once per month
_____ At least 2-3 times per month
_____ At least once per week
_____ Two to three times per week
_____ Four or more times per week (Everyday? _____)

4. What seed types of beans do you consume? Check all that apply:
_____Red mottled
_____Purple speckled
_____Yellows
_____Whites
_____Composites (mixtures of beans)
_____Cranberry
_____Other (Please specify) _____
_____I don't know type

5. What factors influence your choice of bean seed type? Check all that apply:
_____Tradition/ Custom
_____Price
_____Texture
_____Flavor
_____Health/Nutrition
_____Availability

6. Do you grow beans on your farm?
____Yes
____No

____I don’t have a farm
____I rent the land

7. If yes how many acres of beans do you grow per season?
_____ a quarter of an acre
_____ a half of an acre
_____ one acre
_____ two acres
_____ others (Please specify) _____
_____ I don’t know

8. Do you grow **climbing beans** or **bush types**? _____

9. How often do you grow beans?
____Once a year
____Twice a year
____More than twice a year

10. What seed types of bean varieties or seed types do you mainly grow? Check all that apply:

_____Red mottled
_____Purple speckled
_____Yellows
_____Whites
_____Composites (Mixtures of beans)
_____Cranberry (Kanyeebwa types)
_____Other (Please specify) _____

11. What factors influence your choice of bean seed types to grow? Check all that apply:
_____Tradition/ Custom
_____Marketability/ Price
_____Drought tolerance
_____Cooking time
_____Health/Nutrition
_____Seed availability
_____Early maturity
_____Pest and disease resistance
_____Seed size and color
_____Others (Please specify) _____

12. Which other crops do you grow on your farm?
_____ Cassava
_____ Sweet potatoes
_____ Maize
_____ Irish potatoes
_____ Ground nuts
_____ Bananas
_____ Millet
_____ Leafy vegetables
_____ Tomatoes
_____ Others (Please specify) _____

13. Do you sell some of your bean produce? ____Yes _____No

14. If yes, where do you sell your bean produce?
_____ Local traders
_____ Roadside market
_____ Urban traders
_____ Schools
_____ Relief organizations
_____ Prisons
_____ Others (Please specify) _____

15. If yes, where do you sell your bean produce? What is the unit of your sale?
_____ Bag (_____50KG Bag or _____100KG Bag)
_____ Basin
_____ Basket
_____ Tin

16. What energy source do you use to cook beans in your household?
_____ Wood fuel/ Fire wood
_____ Charcoal
_____ Biogas
_____ Kerosene
_____ Electricity
_____ Others (Please specify) _____

17. What activities do you do to prepare beans for cooking in your household? Check all that apply:
_____ Sorting
_____ Soaking
_____ Dehulling

_____ Others (Please list) _____

18. In case you soak beans, for how long do you soak them?
_____ 3 hours
_____ 6 hours
_____ 12 hours
_____ Others (Please specify) _____

19. In case you soak beans or do not soak the beans, for how long do your beans take to cook?

Cook-time without soaking	**Cook-time after soaking**
_____ 1 hour _____ 2 hours _____ 3 hours _____ 6 hours	_____ 1 hour _____ 2 hours _____ 3 hours _____ 6 hours

20. Apart from soaking are there other strategies you use (things you do) to make beans cook faster? Please list if any.

1. _____

2. _____

3. _____

4. _____

Thank You Very Much!!!!!

Appendix E: Survey instrument for sensory evaluation of the high mineral dry bean acceptability study in Uganda

You are provided with a sample of cooked beans. Please observe and record your liking for the appearance and color on the hedonic scale below. Use the provided spoon and place sufficient bean sample in your mouth. Taste the beans and rate them against the given scale by placing a check mark at the appropriate position on the hedonic scale for taste, flavor, texture, and overall acceptability. Please evaluate the products in the order in which they are presented to you. Use the bottled mineral water provided to rinse your mouth before and after tasting each sample and between samples. **PLEASE ANSWER ALL QUESTIONS**. We really would like to know what you think!! **If you have any questions, please ask the coordinators of this study at any time.**

Note: If you have any further comment(s) about the bean samples presented to you, please write the comment(s) on the space provided below at the end of this questionnaire.

Now please look at sample _____
Please LOOK at the sample and answer the following question.
Please DO NOT taste the sample yet.

1. How much do you **LIKE** or **DISLIKE** the **BEAN AND BROTH COLOUR** of this sample?

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

2. How much do you **LIKE** or **DISLIKE** the **OVERALL APPEARANCE** of this bean sample?

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike

- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Next, please now taste the sample and answer the following questions for sample _____

Please feel free to go back and taste the sample again before answering if necessary.

1. How much do you **LIKE** or **DISLIKE** the **OVERALL FLAVOR** of this sample?
   - ☐ Like extremely
   - ☐ Like very much
   - ☐ Like moderately
   - ☐ Like slightly
   - ☐ Neither like nor dislike
   - ☐ Dislike slightly
   - ☐ Dislike moderately
   - ☐ Dislike very much
   - ☐ Dislike extremely

2. How much do you **LIKE** or **DISLIKE** the **TASTE** of this bean sample?
   - ☐ Like extremely
   - ☐ Like very much
   - ☐ Like moderately
   - ☐ Like slightly
   - ☐ Neither like nor dislike
   - ☐ Dislike slightly
   - ☐ Dislike moderately
   - ☐ Dislike very much
   - ☐ Dislike extremely

3. How would you rate this sample on the **TEXTURE OF THE BEANS**?
   - ☐ Much Too Firm
   - ☐ A Little Too Firm
   - ☐ Just About Right
   - ☐ A Little Too Soft
   - ☐ Much Too Soft

4. **Overall** how much do you **LIKE** or **DISLIKE** this bean sample?

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Is there anything else/ comments you would like to tell us about this bean sample?

_____
_____
_____
_____
_____

Thank You Very Much!!!!!