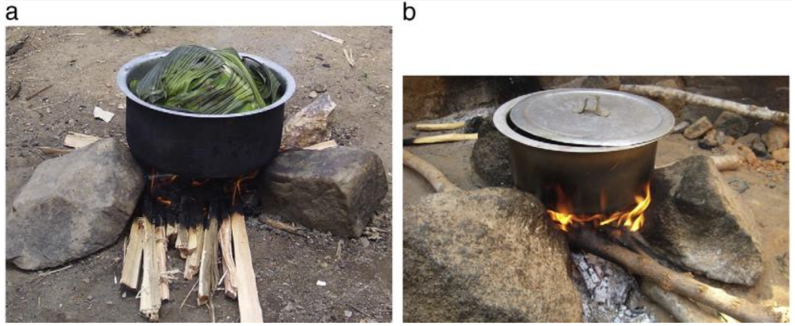
Development and validation of KASP markers associated with cooking time and canning quality traits in common beans (*P. vulgaris)*

Kesiime V. Eunice, Stanley T. Nkalubo, Isaac Dramadri

National Crops Resources Research Institute, NARO

[redacted]



OUTLINE OF THE PRESENTATION

- Background to the constraint; limitations & interventions
- Development of KASP makers & their technical validation
- Biological validation
- Phenotyping for the two traits & results
- Marker segregation & allelic effect on phenotypic means
- Conclusion
- Acknowledgment

INTRODUCTION

- Common beans are such a nutritionally complete food that,
- Protein, carbohydrtes Fe, Mg, Zn, K and fibers all together in high amounts (Kotu *et al*., 2018).
- Despite that, over the years per capita bean consumption in Uganda and worldwide has been almost constant (19kg and 3 respectively)

Key limitations to bean consumption

Limitations	Effects (human & env’t)
▪ Long cooking time ▪ About 40 -76% of world population uses firewood as a source fuel for cooking ▪ 1 kg dry beans requires 7-11kg of firewood to cook (Adkins *et al.*, 2010) ▪ Incomplete combustion of fuelwood → indoor pollutants	• High fuel, water and time for cooking • High expenditure • Deforestation, impact on water bodies • acute lower respiratory infections and other disorders • Affects gender, denying especially women and young girls time to do other activities

INTERVENTIONS TO LONG COOKING TIME

There is need for development of short cooking bean varieties and processed beans which will;

- Lessen fuel wood needs' impact on consumers and the environment
- Reduce mineral loss and improve the nutritional quality of meals

Processed bean products include; cooked beans, precooked beans (dehydrated and frozen), flours, protein concentrates and canned beans.

Limitations of phenotyping for the two traits

- However phenotyping/ Selection for cooking time and canning quality in common bean is labour intensive
- cooking time estimation methods particularly by use of an automated Matson cooker
- has a low throughput, and consumes a lot of water and electricity which makes the process very expensive in terms of resources,
- time and technical expertise especially when screening large samples.

Limitations of phenotyping for the two traits (cont'd)

- Similarly testing for canning quality requires specialized canning facility

- requires approximately 250g of seed for testing, taking a number of seasons for seed multiplication

- or a number of advancements to the next Filial generation if using breeding lines

- In addition, testing for the two traits involves destructive processes where a reasonable amount of seed cannot be recovered especially during the breeding process

Intervention

- MAS could facilitate the evaluation of breeding lines at the seedling stage
- SNP markers are the most widely used in molecular studies
- Due to their low assay cost, high genomic abundance, bi-allelic nature, locus-specificity, low mutation rate
- However, there exists a wide gap between the development of SNP markers and their subsequent application by breeders

SNP databases provide a large amount of SNP information which requires professional bioinformatic analysis to quickly obtain useful SNPs. Thus, need for an easier way of obtaining this information

Advantages of KASP MARKERS

- Among the different uniplex or multiplex platforms for obtaining SNP data,

- The KASP uniplex assay system has gained wide popularity, because of their;

- Ability to combine PCR amplification with fluorescent detection and amenability to high throughput and automation.

- With this background,

Development of primers

- Fifty nucleotide bases flanking the target SNPs on either side

(17 for cooking time, 37 for canning quality and 8 for water absorption)

- Nucleotides were downloaded from the SEQUART AFRICA server and the ones identified by Cichy *et al* 2015 downloaded online

- With support from EiB, Primers were designed from LGC Bioresearch Technologies UK Lab.

- Technical validation/ SNP verification was conducted with one plate (94 samples) upon which we obtained a report for 28 that ranged from good to very good.

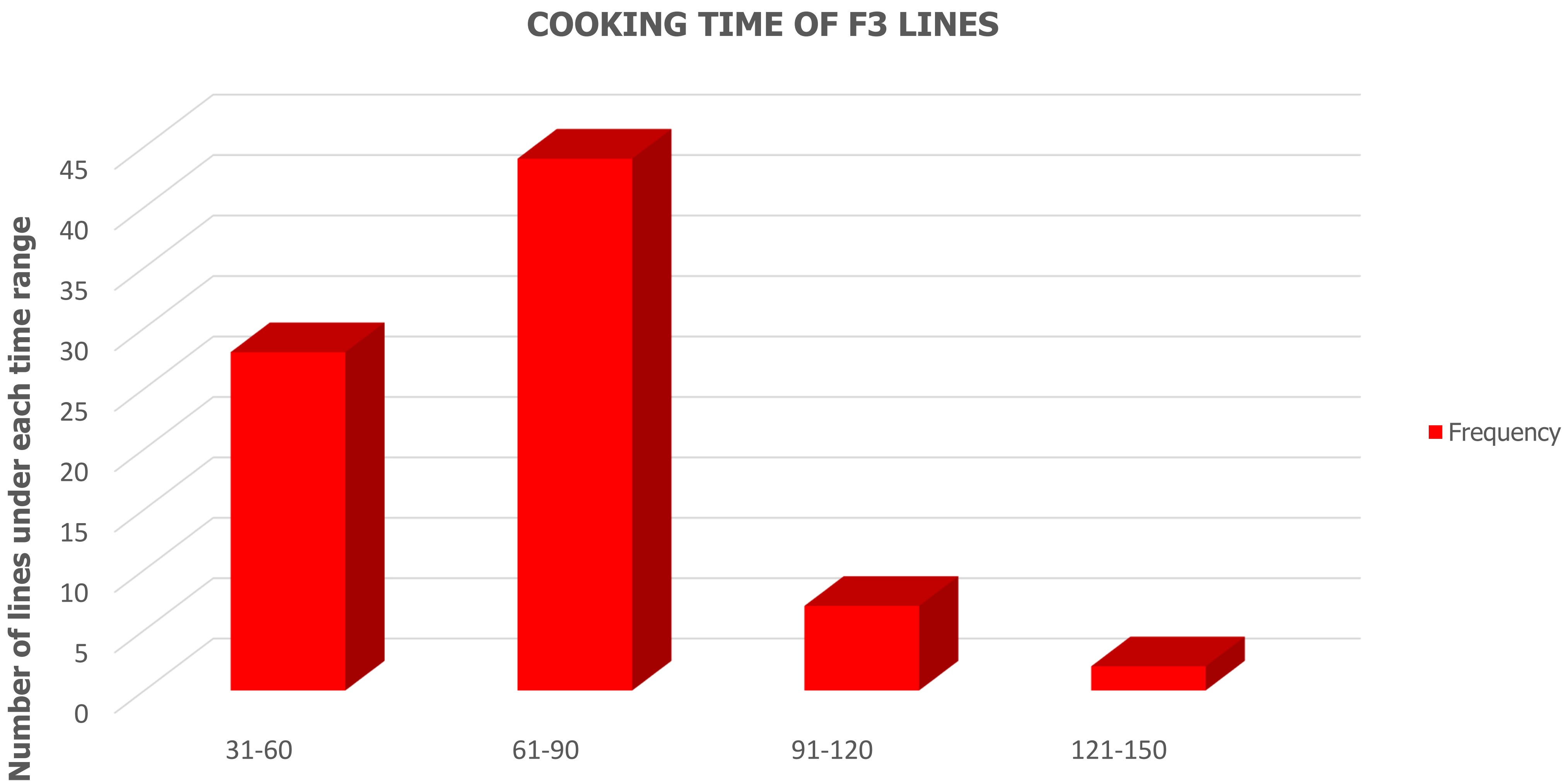
Validation of KASP PCR assays in an independent population

- The assay performance of the twenty-eight KASP markers; cooking time (9), Water absorption (3), splitting (2),
- appearance (2), colour (1), clumping (2), viscosity (4), washed drain solids (3) washed drain coefficient (2) that
- were selected based on SNP verification report, that ranged from good to very good
- assessed using two independent bean populations one for cooking time and the other for canning quality traits independent from that used for GWAS.

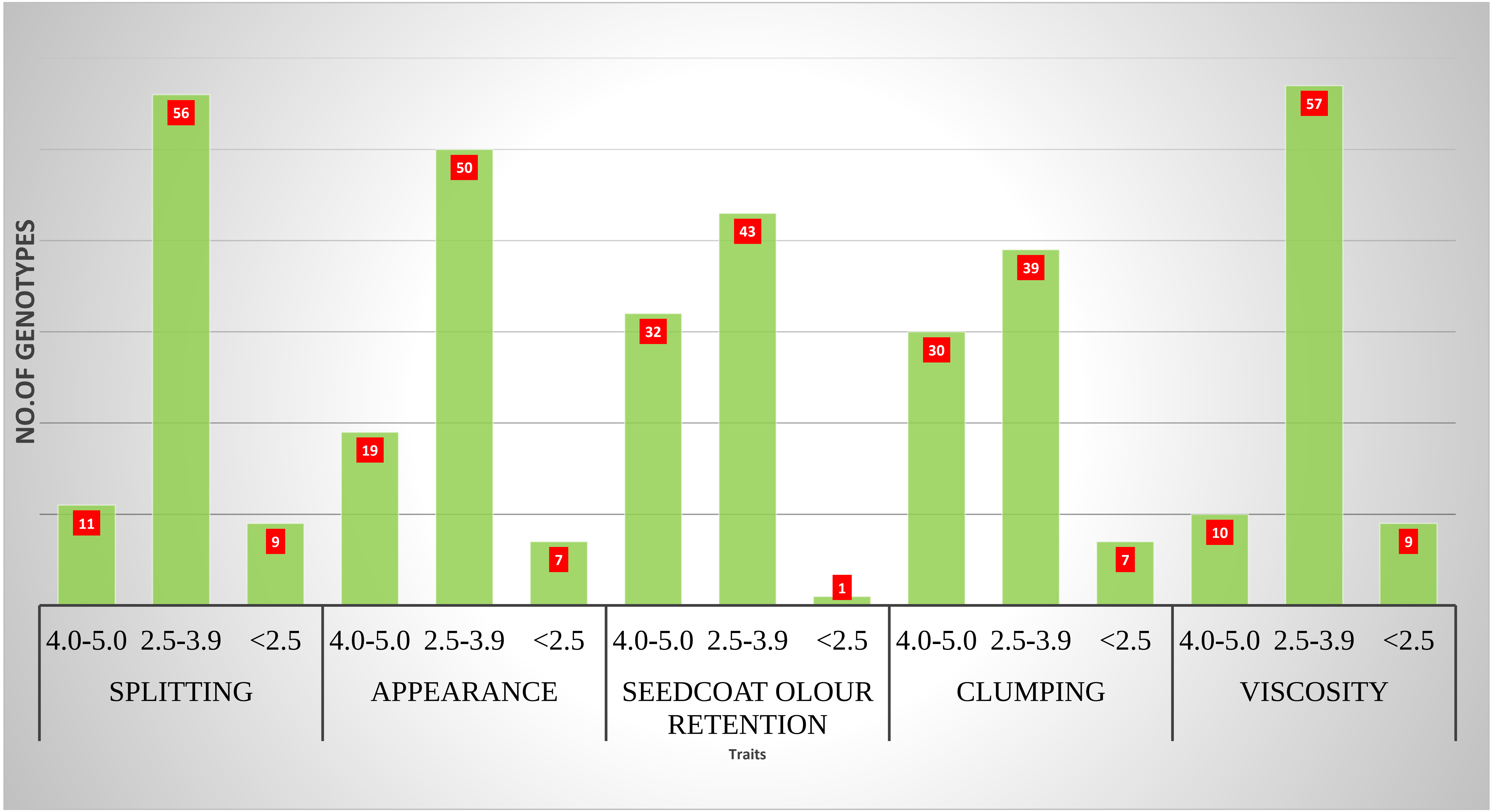
Genotyping

- Leaf discs were cut from the youngest trifoliate leaves of F2 plant populations growing naturally in the field at NaCRRI
- with support from EiB, the bean samples were genotyped with the developed KASP markers at Intertek (Sweden) laboratory
- The genotyped plants were advanced to F_3, F_3 seed was planted in RCBD with 2 rows of 1 meter replicated twice
- F^4 seed was evaluated for canning and cooking time 3 months after harvesting
- CT was estimated- Matson cooker apparatus
- Canned beans were evaluated for; appearance, splitting, colour, viscosity clumping and HC, WDW & WDC calculated

COOKING TIME RESULTS



CANNING QUALITY EVALUATION RESULTS



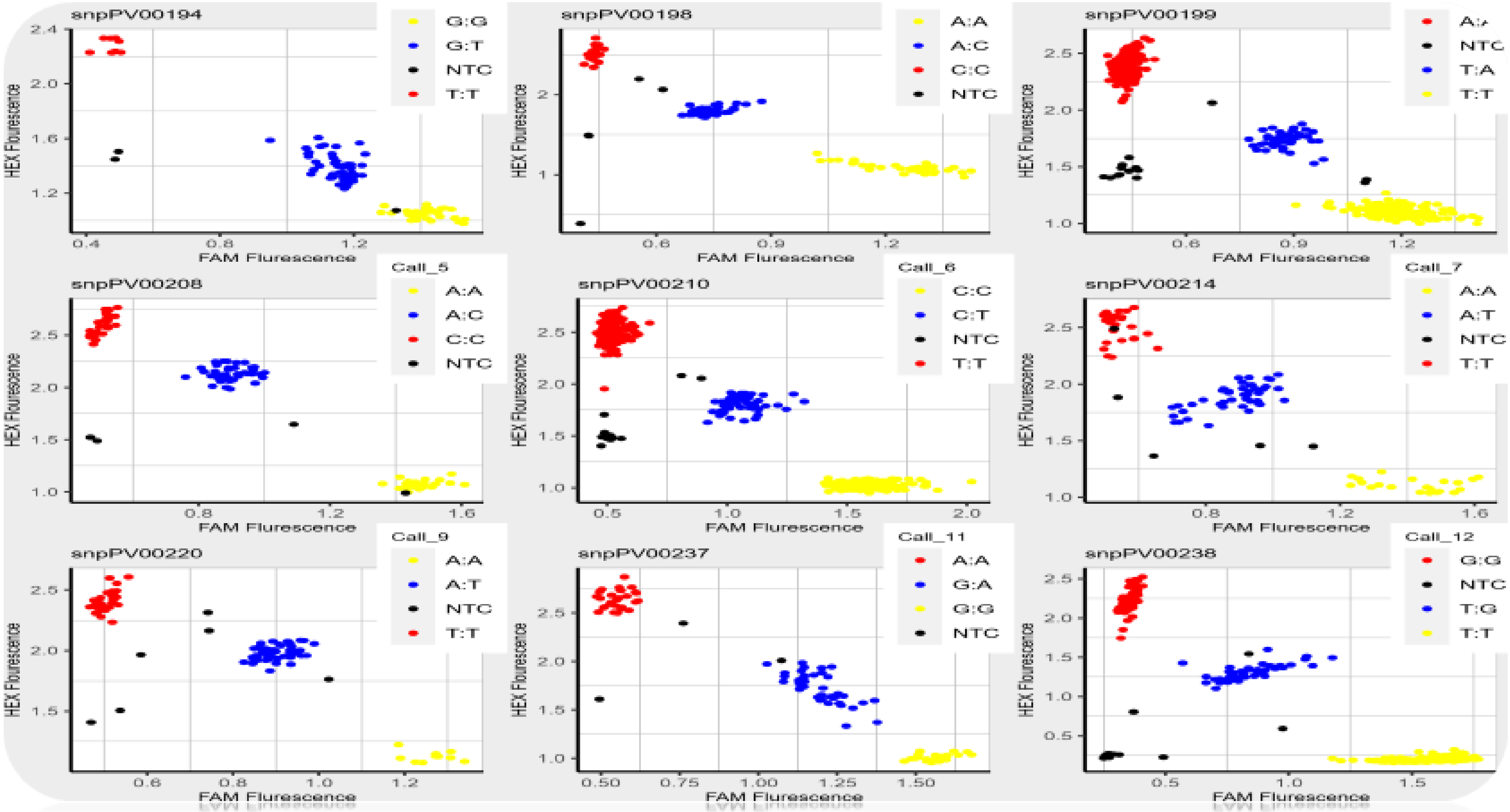
MARKER SEGREGATION AND MARKER EFFECTS

- 7 markers for CT, 11 markers for CQTs and absorption properties clustered into 3 distinct groups
- Homozygous genotypes for the favourable allele, homozygous genotypes for the unfavourable allele and heterozygotes having both alleles.
- The allele frequency for the remaining 10 markers did not follow any consistent pattern, 7 had either only the favourable or the
- unfavourable allele, 2 had both the favourable and heterozygote with no unfavourable allele, 1 for cooking time lacked the heterozygote while one was uncalled.
- There was generally a high call rate (>90%) for 21 markers, 4 above 80% and 2 had a call rate of less than 80%.

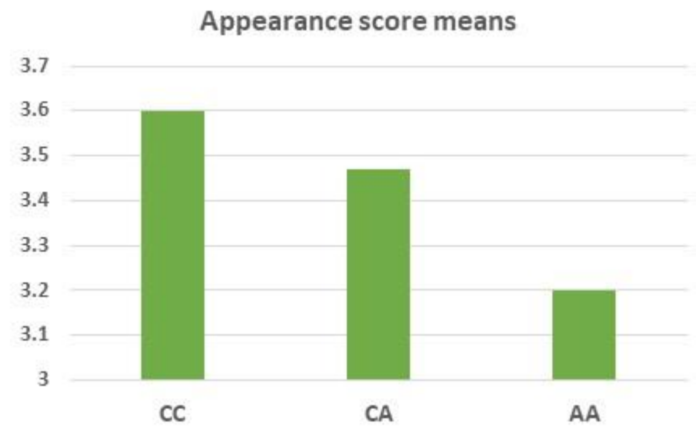
Marker segregation and allelic parameters

TRAIT	MARKER	INTERTAKE. ID	No	%HOM1	%HET	%HOM2	NUL
SPLT	3381147-50-T/G	snpPV00194	94	9.6	53.2	36.2	1.1
APER	3380009-30-C/A	snpPV00198	94	19.1	42.6	35.1	3.2
APER	3375092-60-A/T	snpPV00199	94	23.4	44.7	28.7	3.2
VISC	3373587-14-C/A	snpPV00208	94	29.8	43.6	24.5	2.1
VISC	3383473-23-T/C	snpPV00210	94	17	48.9	31.9	2.1
WDS	100045471-42-T/A	snpPV00214	94	28.7	45.7	22.3	3.2
WDC	3378758-35-T/A	snpPV00220	94	30.9	54.3	10.6	4.3
CT	ss715647434 (A/G)	snpPV00226	94	85.1	7.4	2.1	5.3
CT	ss715646002 (T/C)	snpPV00228	94	86.2	7.4	2.1	4.3
CT	ss715648837 (T/C)	snpPV00229	94	3.2	6.4	86.2	4.3
CT	ss715650437 (T/C)	snpPV00230	94	3.2	6.4	85.1	5.3
CT	ss715640782 (T/C)	snpPV00234	94	89.4	2.1	4.3	4.3
CT	ss715642453 (T/C)	snpPV00235	94	89.4	2.1	4.3	4.3
WA	ss715639608(A/G)	snpPV00237	94	30.9	46.8	21.3	1.1
WA	ss715639606 (T/G)	snpPV00238	94	20.2	44.7	31.9	3.2

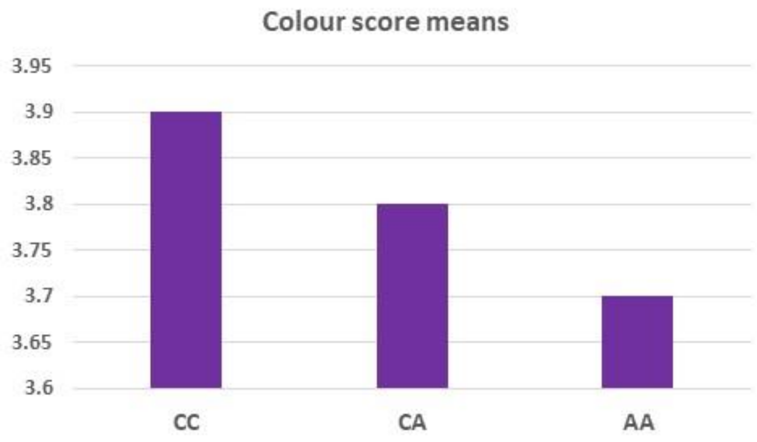
Scatter plot for selected KASP assays showing clustering of genotypes on the Y- and X-axes.



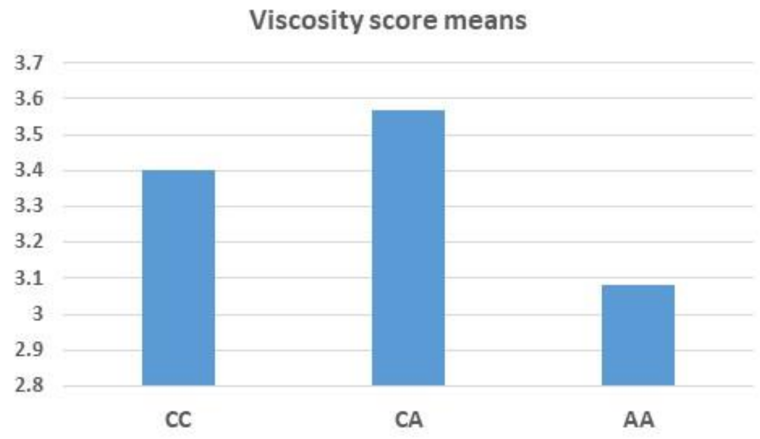
Allelic effects on the mean phenotypic values



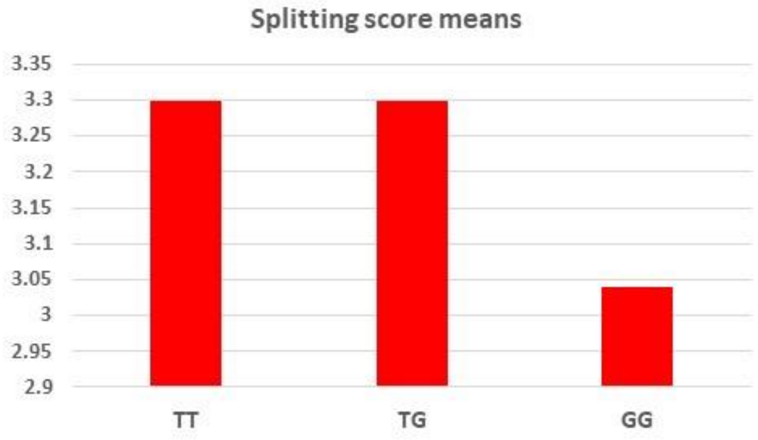
3380009-30-C/A



3380009-30-C/A

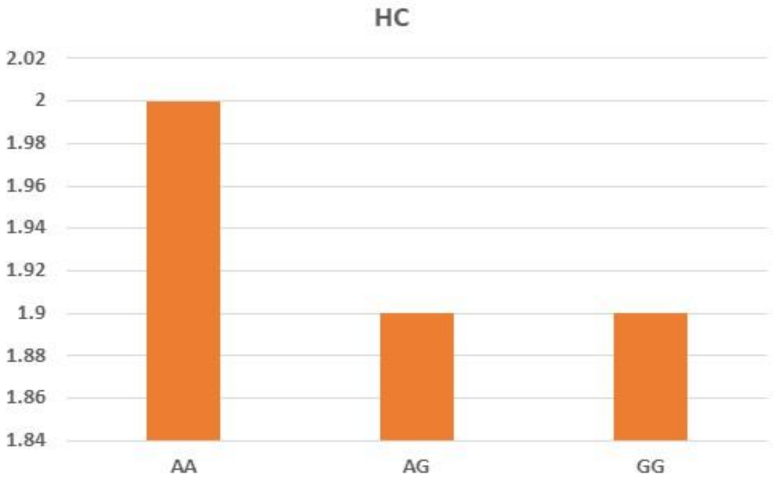


3373587-14-C/A



3381147-50-T/G

Allelic effects on the mean phenotypic values



Ss715639608 (A/G)

100045471-42-T/A

Summary

- This study generated 28 lines that cooked in less than one hour and 35 lines whose total canning score was 70% above.
- 15 KASP essays developed in this study were effective in discriminating among the different allelic states of genotypes at F2
- The genotypes that cooked in < hr, had atleast a copy of the favourable allele for markers ss715647434 (A/G), ss715646002 (T/C), ss715640782 (T/C), ss715642453 (T/C) or the unfavourable allele for markers ss715648837 (T/C) & ss715650437 (T/C)
- For canning quality traits, genotypes that had atleast a copy of the favourable allele for markers; 3380009-30-C/A, 3381147-50-T/G, 3373587-14-C/A & ss715639608(A/G) gave higher means
- Implying that these markers could be strongly linked with genes controlling these traits and thus high potential for application in MAS.

ACKNOWLEDGMENT

CGIAR
Excellence in Breeding
PLATFORM

Makerere University Regional Centre for Crop Improvement
Research and Training for Africa
MaRCCI

Alliance
Bioversity International
CIAT

FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

NARO

NaCRRI
NATIONAL CROPS RESOURCES RESEARCH INSTITUTE

ebca
Enhancing breeding capacity in Africa

Thank you for listening