



Phenotypic Variation, Broad-Sense Heritability and Interrelationship between Grain Yield, Nutritional Traits and Milling Quality in South African Maize Hybrids

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Abstract

Maize is a staple food crop that can provide multiple dietary components, and has the potential to improve food security and address malnutrition. The objectives of this study were to determine the phenotypic variation among maize hybrids for grain yield, nutritional quality traits and milling quality, to identify superior maize hybrids, and to determine the interrelationship between measured traits. Eighteen maize hybrids (nine commercial and nine experimental) were planted in a randomised complete block design with six replicates in seven different environments in South Africa. Genotype, environment and their interaction effects were significant for grain yield and measured traits, indicating the existence of variability in maize breeding populations. Broad sense heritability for starch, milling quality, fibre and defective grain (DEFG) was above 50%, which indicated that the phenotypic differences of these traits were mostly attributed by environmental factors. Grain yield, protein, moisture and fat had broad sense heritability that was below 50%, indicating that the phenotypic differences of the traits were due to genotypic effects. Superior maize hybrids, G15-Ex (grain yield, fat and milling quality), G16-Ex (protein), G11-Ex (starch) and G14-Ex (fibre) were identified. Hybrids G2-C and G4-Ex had low values for DEFG. A significant and positive correlation was observed for protein with grain yield. Milling quality was positively correlated with almost all traits measured, indicating the possibility of multiple trait selection. Starch was negatively associated with protein and grain yield. The clustered heat map distinguished three distinct clusters of maize hybrids namely; 1) G1-C, G7-C, G9-C, G13-Ex, G14-Ex, G16-Ex and G17-Ex associated with protein and fibre, 2) G4-Ex, G5-C, G6-C, G8-C and G11-Ex associated with grain yield, fat, moisture and fibre and, 3) G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex associated with milling quality and fat. High-yielding maize hybrids with good nutritional quality can be tested for adaptability and stability and recommended for commercial release.

Keywords: *Zea mays*; Diversity; Hybrid Breeding; Phenotypic Correlations; Grain Yield; Nutritional Quality

Abbreviations

ASL: Above Sea Level; DF: Degrees of Freedom; E: Environment; G: Genotype; GE Interaction: Genotype by Environment Interaction; F_E : F-value for the Environment; F_G : F-value for Genotypes; F_{GE} : F-value for Genotype by Environment Interaction; σ_G^2 : Genotypic Variance; σ_{GE}^2 : Genotype by Environment Interaction Variance; σ_p^2 : Phenotypic Variance; H^2 : Broad Sense Heritability; SD: Phenotypic Standard Deviation; Gs: Predicted Selection Gains; %Gs: Percent Predicted Selection Gain; Gr: Mean: Grand Mean; DEFG: Defective Grain; PC: Principal Component; PCA: Principal Component Analysis; C: Commercial Hybrids; Ex: Experimental Genotypes; MC: Moisture Content

Introduction

Maize (*Zea mays* L.) is a primary food source for millions of people in sub-Saharan Africa, particularly in Southern Africa where it contributes approximately 15 to 56% of daily caloric intake [1]. Maize has high nutritional value, its grains contain 72 to 73% carbohydrate i.e., amylose (25 to 30%) and amylopectin (70 to 73%), 6 to 20% protein, 2 to 4% fat, vitamins (B6, A and E) and essential minerals (Fe and Zn) [2]. The crop also contains 0.03 to 1.87% fibre which is responsible for water retention in humans during food digestion [3,4]. Although maize protein is deficient in lysine and tryptophan, it contains considerable amounts of methionine and cysteine. Due to its nutritional composition, the crop is mainly produced for human consumption as food and as an animal feed.

Maize is grown in diverse environments with varying temperatures, soil types and rainfall, making it a suitable crop for climate smart agriculture [5]. Researchers have been developing climate smart maize hybrids with high grain yield, improved nutritional quality and other desired traits [6,7]. Previous studies have indicated that grain yield and most of the nutritional quality traits are polygenic with small additive genetic effects [8]. Polygenic traits are mainly influenced by environmental factors, resulting in low broad sense heritability estimates, which ultimately reduces selection efficiency [9]. Thus, comprehensive and ongoing research that focuses on investigating the influence of genotype and environmental conditions on the phenotypic expression and heritability of grain yield, nutritional quality traits and milling quality is required [10].

Genotypic and phenotypic variance as well as heritability are the most important parameters that determine the selection efficiency in maize breeding programmes [11]. Genotypic variance quantifies the portion of the phenotypic variance among genotypes in diverse environments [10]. Whereas phenotypic variance explains the total variation among genotypes in different environments. Both genotypic and phenotypic variance are used to determine the broad-sense heritability. Broad-sense heritability is the amount of phenotypic variance that is attributable to the overall genetic variance of the populations. It quantifies the value of selection for a specific characteristic in different types of progenies [12].

Previous studies conducted in Ethiopia [9,13], Nigeria [14,15], Zimbabwe [16], Kenya [17], Ghana [18], Uganda [19], India [20] and South Africa [21,22], focused on the assessment of phenotypic diversity of maize hybrids in various environments for grain yield and yield components and to some extent on nutritional composition, and reported significant variation among the tested populations.

Multiple trait selection is required for developing maize hybrids with the desired characteristics and to understand how improving one trait would influence other traits [1]. Phenotypic correlation is one of the methods used to study relationships between traits in a population [23]. In a maize breeding programme, phenotypic correlations between pairs of variables give opportunities for simultaneous selection [24,25]. Other methods such as principal component analysis and clustered maps have been used to assess the interrelationships among maize phenotypic and nutritional characteristics and their association with genotypes [26-28].

Therefore, the evaluation of the phenotypic diversity of maize hybrids for grain yield, nutritional quality characteristics and milling index is important in order to determine the variation present in the available material, which will ultimately enable the identification and selection of superior hybrid genotypes. In addition, understanding the interrelationship between the traits associated with superior hybrid genotypes is expected to guide and improve the selection efficiency. Hence, the objectives of the study was to determine the phenotypic variation among maize hybrids for grain yield, nutritional quality traits and milling quality, to identify superior maize hybrids, and to determine the interrelationship between measured traits.

Materials and Methods

Study material and experimental environments

Eighteen maize genotypes (nine commercial hybrids: G1-C, G2-C, G3-C, G5-C, G6-C, G7-C, G8-C, G9-C and G10-C and nine experimental hybrids: G4-Ex, G11-Ex, G12-Ex, G13-Ex, G14-Ex, G15-Ex, G16-Ex, G17-Ex and G18-Ex) were obtained from a private seed company in South Africa. All maize hybrids were obtained from the final stage of the breeding programme (the stage before commercial release). Maize hybrids were planted in seven different environments during the 2020/2021 cropping season. The experimental sites represented different environmental conditions and were located in the eastern part of the maize production areas of South Africa (Table 1).

Environment	Environment (E) code	Latitude (S)	Longitude (E)	Altitude ASL (m)	Average seasonal max temperature (°C)	Average seasonal min temperature (°C)	Average seasonal rainfall (mm)
Bethal	E1	26°46'	29°47'	1661	31	2	478
Leandra	E2	26°37'	28°92'	1687	32	1	616
Middleburg	E3	25°46'	29°27'	1479	27	5	958
Wonderfontein	E4	25°80'	28°88'	1459	33	-3	421
Petit	E5	26°09'	28°39'	1649	32	-1	649
Kriel	E6	26°27'	29°23'	1552	33	2	616
Amersfoort	E7	26°89'	29°85'	1652	31	0	705

Table 1: Description of the experimental sites.

ASL = Above sea level.

Experimental design, trial establishment and management

The field trials were laid out in a randomized complete block design with six replications. The experimental plots consisted of four rows of 6 to 12 m long with a spacing 0.75 m between rows and 0.25 m between plants. All trials were planted using private seed company plot planters and managed by commercial farmers. Fertilizer, weeding and harvesting were performed as per the recommendations for each environment.

Data collection

Grain weight (kg) and moisture content (%) was collected using the combine harvester from the two inner rows in the four-row plot and converted to ton per hectare. The following formula was used to calculate grain yield:

$$\text{Grain yield} = \left(\frac{\text{grain weight} \times 10 \times (100 - \text{MC})}{(100 - \text{adjusted MC}) (\text{plot area})} \right)$$

MC = moisture content

Nutritional quality traits

Two sub-samples for each sample were used to determine the milling index (%), which is the indication of the milling ability and milling quality of maize kernels and nutritional quality traits such as starch (%), protein (%), moisture (%), fat (%), and fibre content (%) using near-infrared spectroscopy (NIR) (a Perten Grain Analyzer, Model DA 7250, Perten, Instruments AB, Sweden) with a wavelength ranging from 900 to 1700 nm.

Defective grain (DEFG)

After thorough mixing, a random 100 g sample of maize grain per hybrid was weighed and sieved manually using a 6.35 mm round-hole sieve. Maize grains that remained above the sieve were classified as damaged kernels, which could have been due to mould, insect or rodent damage, water damage and pinking. Kernels that passed through the sieve were weighed separately as 'under sieve'. These were then used to calculate DEFG as a percentage of the total 100 g sample per hybrid.

Statistical analysis

Combined analysis of variance (ANOVA) were done for grain yield, nutritional quality traits and milling quality using GenStat version 2021 [29] and SAS software [30], and least significant differences at a 5% α -level were used to separate the means for measured traits. The genotypes (hybrids) were considered fixed because they represented all advanced maize hybrids before commercial release. Environments represented a random sample of all possible environments that represented maize growing environ-

ments in the eastern parts of South Africa. Genotypic and phenotypic variances were calculated from the mean squares generated from ANOVA [31].

Genotype, genotype by environment (GE) interaction and phenotypic variances for combined analysis were calculated using the following formulae:

$$\sigma_g^2 = \text{genotypic variance} = MS_G - MS_{GE} / re \dots\dots\dots 4$$

$$\sigma_{GE}^2 = \text{genotype by environment interaction variance} = MS_{GE} - MS_{error} / r \dots\dots\dots 5$$

$$\sigma_p^2 = \text{phenotypic variance} = \left(\frac{\sigma_{error}^2}{re} + \frac{\sigma_{GE}^2}{e} + \sigma_g^2 \right) \dots\dots\dots 6$$

Where MS_G = mean squares of genotypes, MS_{GE} = mean squares of genotype by environment interaction, MS_e = mean squares of error, e = environments, and r = replications.

Broad sense heritability (H^2) was calculated using the following formulae:

$$H^2 = \sigma_g^2 / \sigma_p^2 \dots\dots\dots 7$$

Predicted selection gains (Gs) was calculated using the following formulae:

$$\%Gs = \frac{k \times \sigma_p \times H^2}{\text{Grand mean}} \times 100 \dots\dots\dots 8$$

Where %Gs = percent predicted selection gain, k = selection intensity at 5%, σ_p = phenotypic standard deviation, and H^2 = broad sense heritability.

Phenotypic correlations for grain yield, nutritional quality traits and milling quality were estimated using SAS software [30]. The principal component analysis (PCA) was done to visualize the performance of maize genotypes in relation to specific traits using XLSTAT 2022 [32]. A clustered heat map was generated to visualize the hierarchy of clusters among maize genotypes for grain yield, nutritional quality traits and milling quality using NCSS [33].

Results

Combined ANOVA

Genotype and GE interaction effects were highly significant ($P \leq 0.001$) for grain yield, all nutritional quality traits and milling quality (Table 2). Phenotypic variances were higher than genotypic variances for all traits. H^2 estimates were high for milling quality (82.50%), fibre (78.57%), starch (60.61%) and DEFG (58.25%) but low for grain yield (17.63%), moisture content (30.86%), fat (38.19%) and protein (46.58%). Starch (1.07%) and moisture (2.18%) had the lowest %Gs while DEFG (92.80%) had the highest.

Source of Variation	DF	Grain yield (ton/ha)	Protein (%)	Starch (%)	Fibre (%)	Fat (%)	Moisture (%)	Milling quality (%)	DEFG (%)
E	5	1021.27***	50.52***	566.86***	0.37***	5.41***	20.26***	1821.66***	272.94***
Block (E)	30	3.9	0.44	0.93	0.01	0.4	0.15	15.13	31.46
G	17	3.29*	1.43***	3.57***	0.06***	1.04***	0.29*	1282.73***	152.39***
GE interaction	85	2.71***	0.76***	1.41***	0.02***	0.64***	0.20*	224.99***	63.40***
Error	510	1.62	0.32	0.83	0.01	0.31	0.14	15.07	21.94
Total	647	6332.08	499.56	841.4	8.23	254.79	203.6	64687.1	21658.5
F_E		260.71***	123.67***	54.41***	57.19***	15.17***	147.57***	130.12***	8.56***
F_G		2.04*	4.42***	2.78***	8.10***	3.35***	1.97*	85.67***	7.33***
F_{GE}		1.67***	2.24***	1.72***	2.95***	2.06***	1.38*	14.97**	2.9***
σ^2_G		0.0101	0.027	0.0659	0.0012	0.028	0.005	29.465	6.7606
σ^2_{GE}		0.1884	0.0673	0.0881	0.0025	0.06	0.01	34.9867	8.4545
σ^2_P		0.0865	0.0471	0.1036	0.0019	0.0466	0.0106	35.7147	8.7791
H^2 (%)		17.63	46.85	60.61	78.57	38.19	30.86	82.5	58.25
Gr. mean \pm SD		6.77 \pm 1.27	6.84 \pm 0.57	66.89 \pm 0.91	2.48 \pm 0.08	4.30 \pm 0.56	11.02 \pm 0.37	69.79 \pm 3.88	6.06 \pm 4.68
Gs		0.46	0.55	1.14	0.13	0.44	0.24	6.6	5.62
%Gs		6.79	8.04	1.7	5.24	10.23	2.18	9.46	92.8

Table 2: Combined ANOVA for maize grain yield, nutritional quality traits and milling quality.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, DF = Degrees of freedom, E = Environment, G = Genotype, GE interaction = Genotype by environment interaction, F_E = F-value for the environment, F_G = F-value for genotypes, F_{GE} = F-value for genotype by environment interaction, σ^2_G = Genotypic variance, $\sigma^2_{G \times E}$ = Genotype by environment interaction variance, σ^2_P = Phenotypic variance, H^2 = Broad sense heritability, SD = Phenotypic standard deviation, Gs = Predicted selection gains, %Gs = Percent predicted selection gain, Gr. mean = Grand mean, DEFG = Defective grain.

Combined analysis mean values

The genotypes showed large variation across the environments for all traits, ranging from 6.47 to 7.25 ton/ha for grain yield, 6.20 to 7.36% for protein content, 66.32 to 67.77% for starch content, 2.41 to 2.60% for fibre, 4.02 to 4.90% for fat, 10.78 to 11.25% moisture, 54.98 to 83.47% milling quality and 3.34 to 16.80% for DEFG (Table 3). On average, hybrid G15-Ex had the highest grain yield (7.25 ton/ha), fat content (4.90%) and milling quality (83.47%), hybrid G16-Ex had the highest protein content (7.36%) and starch content (66.77%) as well as the lowest moisture content (10.78%), hybrid G14-Ex had the highest fibre content (2.60%), while G2-C and G4-Ex had the lowest DEFG (3.34%).

Phenotypic correlations

Highly significant ($P \leq 0.001$) and positive correlations were observed for milling quality with low DEFG ($r = 0.217$) (Table 4). Highly significant ($P \leq 0.001$) and negative correlations were found for protein with starch ($r = -0.636$), fibre with milling quality ($r = -0.411$), low moisture with starch ($r = -0.341$) and fibre ($r = -0.334$), fat with protein ($r = -0.308$).

Principal component analysis (PCA) for grain yield, nutritional quality traits and milling quality

The eight measured traits were reduced to three principal components (PC) that showed 75.13% of the overall variability observed in the dataset when a minimum threshold eigenvalue of one was used (Table 5). Only PC1 and PC2 were interpreted because they accounted for the majority of the variation in the data set. Milling quality, fat, and moisture contributed the most to PC1 (positively) and protein and fibre (negatively). Grain yield and protein had a significant and positive influence on PC2, whereas starch had a negative influence (Table 5).

The PCA was used to visualise genotype and trait associations (Figure 1). PC1 was plotted against PC2 to distinguish maize hybrid genotypes according to their associated traits. Maize hybrid genotypes G2-C, G4-Ex, G10-C, G11-Ex, G12-Ex, G15-Ex and G18-Ex were on the positive side of PC1 suggesting that these genotypes had high values for milling quality, fat and moisture and low values for protein and fibre. Maize hybrids G7-C, G9-C, G13-Ex and G14-Ex were displayed on the positive side of PC2 indicating that these

hybrids had high values for grain yield and protein and low values for starch content. The PCA further distinguished four different groups of hybrid genotypes according to their associated traits, namely, I) G11-Ex and G12-Ex were associated with high starch content, II) G2-Ex, G4-Ex, G10-C, G15-Ex and G18-Ex were associated with high milling quality, fat, moisture and DEFG, III) G1-C, G3-C, G5-C, G6-C, G8-C, G16-Ex and G17-Ex were associated with

high protein and grain yield, and IV) G7-C, G9-C, G13-Ex and G14-Ex were associated with high fibre. The PCA also showed that milling quality, fat, moisture and DEFG were positively correlated, and all these traits were negatively correlated with fibre. Protein was positively correlated with grain yield and these traits were negatively correlated with starch.

Genotypes	Grain Yield (ton/ha)	Protein (%)	Starch (%)	Fibre (%)	Fat (%)	Moisture (%)	Milling quality (%)	DEFG (%)
G1-C	7.15 ± 3.49	7.09 ± 0.99	66.50 ± 1.14	2.50 ± 0.12	4.02 ± 0.57	10.93 ± 0.52	68.74 ± 6.27	5.50 ± 4.46
G2-C	6.74 ± 3.15	6.70 ± 0.87	67.10 ± 1.08	2.41 ± 0.09	4.43 ± 0.80	11.12 ± 0.57	80.67 ± 8.61	3.34 ± 2.58
G3-C	6.75 ± 3.09	7.30 ± 1.01	67.02 ± 1.42	2.43 ± 0.09	4.12 ± 0.63	11.03 ± 0.58	76.75 ± 4.06	4.45 ± 3.60
G4-Ex	6.91 ± 3.22	6.68 ± 0.91	66.55 ± 1.31	2.44 ± 0.07	4.49 ± 0.61	11.23 ± 0.56	65.23 ± 3.52	3.34 ± 2.21
G5-C	7.09 ± 3.35	6.80 ± 1.01	66.50 ± 1.28	2.49 ± 0.13	4.44 ± 0.54	11.10 ± 0.66	69.13 ± 11.57	5.15 ± 5.84
G6-C	7.14 ± 3.19	6.66 ± 0.90	66.86 ± 1.12	2.48 ± 0.11	4.45 ± 0.58	11.03 ± 0.64	67.14 ± 6.03	3.69 ± 2.52
G7-C	6.94 ± 3.03	6.85 ± 0.81	67.24 ± 1.13	2.47 ± 0.11	4.05 ± 0.66	10.92 ± 0.70	75.49 ± 4.55	8.03 ± 7.43
G8-C	7.11 ± 3.19	6.66 ± 0.98	66.82 ± 1.04	2.47 ± 0.11	4.41 ± 0.63	10.96 ± 0.63	62.99 ± 4.37	5.36 ± 7.54
G9-C	6.79 ± 3.18	7.01 ± 0.87	67.07 ± 1.32	2.49 ± 0.11	4.05 ± 0.42	10.96 ± 0.51	68.95 ± 7.13	8.45 ± 6.51
G10-C	6.56 ± 3.13	6.68 ± 0.79	67.04 ± 1.10	2.44 ± 0.10	4.44 ± 0.75	10.95 ± 0.48	76.60 ± 7.78	9.08 ± 9.08
G11-Ex	6.80 ± 3.15	6.52 ± 1.09	67.77 ± 1.25	2.48 ± 0.11	4.12 ± 0.54	11.05 ± 0.60	66.09 ± 8.48	4.05 ± 2.98
G12-Ex	5.53 ± 3.62	6.20 ± 0.57	67.70 ± 0.89	2.45 ± 0.14	4.66 ± 0.70	11.07 ± 0.73	80.70 ± 7.56	7.50 ± 7.45
G13-Ex	6.59 ± 3.03	6.94 ± 0.81	66.85 ± 1.00	2.51 ± 0.11	4.47 ± 0.84	11.15 ± 0.54	63.52 ± 4.10	3.77 ± 4.01
G14-Ex	6.56 ± 3.07	6.75 ± 0.91	66.84 ± 0.97	2.60 ± 0.16	4.11 ± 0.52	10.87 ± 0.56	54.98 ± 10.45	4.06 ± 4.22
G15-Ex	7.25 ± 2.75	6.76 ± 0.92	66.66 ± 1.21	2.44 ± 0.11	4.90 ± 0.91	11.25 ± 0.67	83.47 ± 7.59	16.80 ± 9.76
G16-Ex	6.64 ± 3.30	7.36 ± 1.20	66.69 ± 1.42	2.51 ± 0.16	4.03 ± 0.58	10.78 ± 0.50	66.51 ± 11.06	7.69 ± 4.48
G17-Ex	6.47 ± 3.40	7.01 ± 0.67	66.32 ± 1.32	2.51 ± 0.09	4.16 ± 0.64	10.95 ± 0.61	63.70 ± 10.15	4.54 ± 4.39
G18-Ex	6.47 ± 3.29	6.96 ± 1.03	66.57 ± 1.21	2.41 ± 0.09	4.25 ± 0.48	11.22 ± 0.64	71.62 ± 12.57	4.45 ± 3.17
Minimum	6.47	6.2	66.32	2.41	4.02	10.78	54.98	3.34
Maximum	7.25	7.36	67.77	2.6	4.9	11.25	83.47	16.8
LSD	0.56	0.21	0.3	0.02	0.20	0.12	1.21	0.12

Table 3: Combined mean values ± standard deviation of 18 maize hybrids for grain yield (ton/ha), nutritional quality traits and milling quality.

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, DEFG = Defective grain.

Traits	Grain yield	Protein	Starch	Fibre	Fat	Moisture	Milling quality
Protein	0.173**						
Starch	-0.134**	-0.636***					
Fibre	0.191	0.085*	-0.123**				
Fat	-0.071	-0.308***	-0.174***	-0.117**			
Moisture	-0.163**	0.040	-0.341***	-0.334***	-0.020		
Milling quality	0.047	0.002	0.044	-0.411***	0.101*	0.044	
DEFG	0.034	-0.112**	0.149***	-0.100*	0.129**	-0.053	0.217***

Table 4: Phenotypic correlation of maize grain yield, nutritional quality traits and milling quality.

* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, DEFG = Defective grain.

Traits	PC1	PC2	PC3
Grain yield	-0.10	0.57	-0.09
Protein	-0.33	0.42	0.36
Starch	0.20	-0.59	0.26
Fibre	-0.43	-0.16	-0.11
Fat	0.46	0.11	-0.31
Moisture	0.41	0.21	-0.46
Milling quality	0.46	0.08	0.46
DEFG	0.25	0.25	0.52
Eigenvalue	3.10	1.71	1.20
Individual%	38.81	21.37	14.94
Cumulative%	38.81	60.19	75.13

Table 5: Principal component (PC) analysis for grain yield, nutritional quality traits and milling quality.

DEFG = Defective grain.

Clustered heat map for grain yield, nutritional quality traits and milling quality

The clustered heat map rows reflect hybrid genotypes, while the columns represent measured traits (Figure 2). Yellow, orange and red represent positive Z-score values above the mean. Green and blue represent Z-score values below the mean. Three distinctive clusters for traits were observed, namely, 1) grain yield, fat and moisture, 2) starch, milling quality and DEFG and, 3) protein and fibre. The clustered heat map also grouped hybrid genotypes into three distinctive clusters namely, 1) G1-C, G7-C, G9-C, G13-Ex, G14-Ex, G16-Ex and G17-Ex were positively associated with high protein and fibre and negatively associated with fat and moisture, 2) G4-Ex, G5-C, G6-C, G8-C, G11-Ex were positively associated with high grain yield, fat, moisture and fibre and negatively associated with protein, milling quality and DEFG and 3) G2-C, G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex were positively associated with milling quality and fat and were negatively associated with protein, fibre and grain yield.

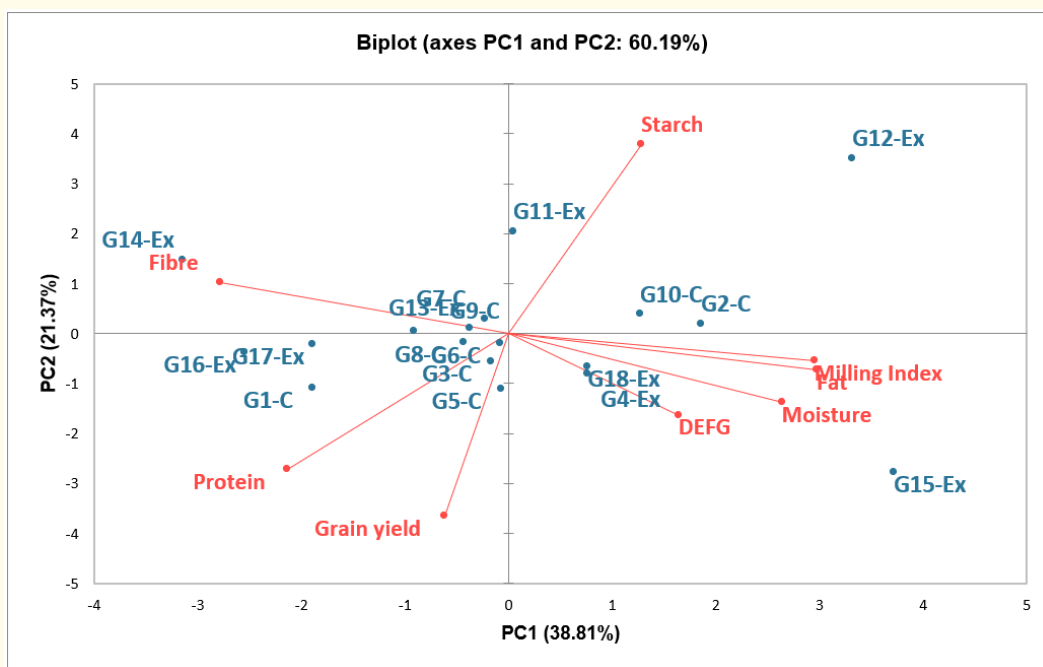


Figure 1: Principal component (PC) analysis biplot of 18 genotypes for grain yield, nutritional quality traits and milling quality. DEFG = defective grain. Genotypes with Ex were experimental hybrids while genotypes with C were commercial hybrids.

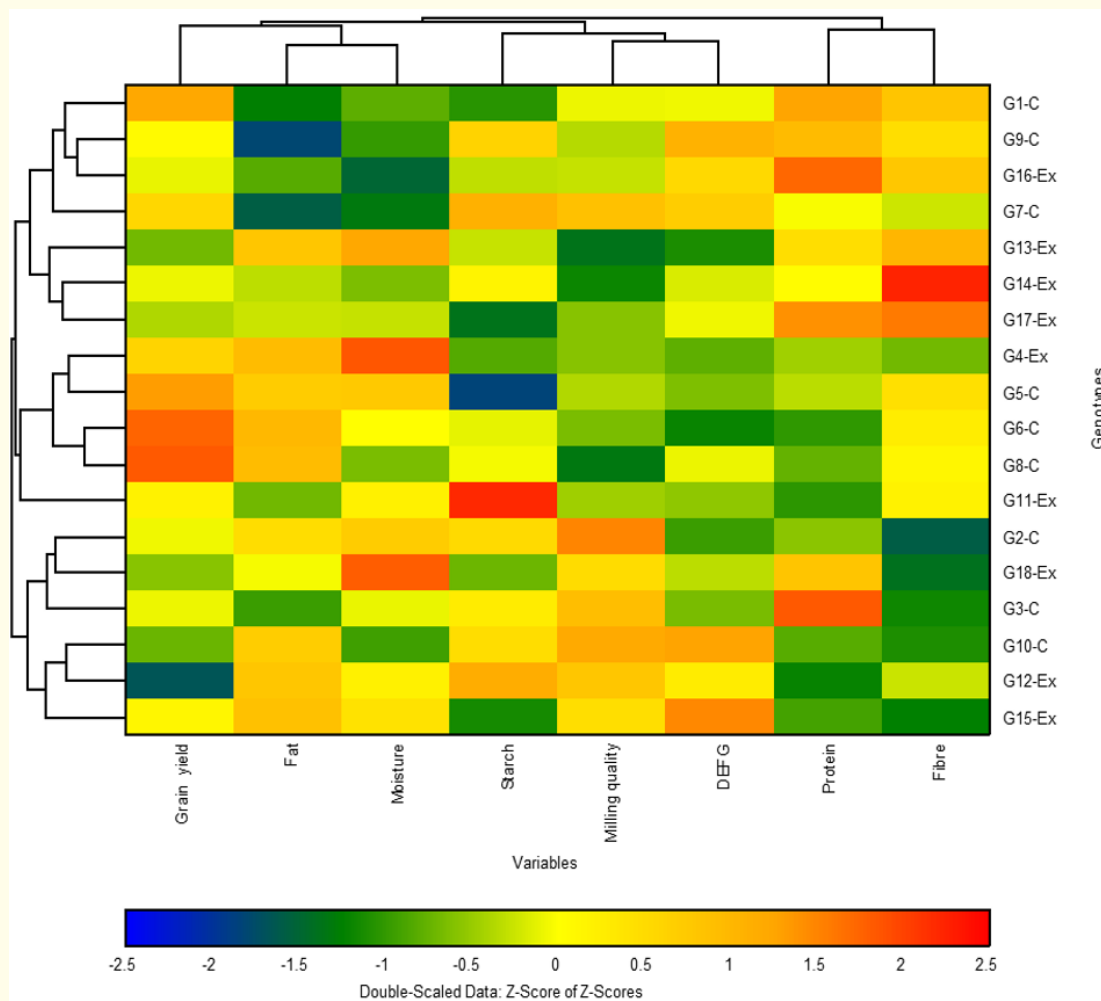


Figure 2: Clustered heat map illustrating associations between maize hybrids for grain yield, nutritional quality traits and milling quality. DEFG = Defective grain.

Discussion

Significant differences among genotypes (hybrids) for all traits suggested the presence of genetic variation among maize hybrids. This suggests that high performing hybrids can be selected. Significant differences among 88 maize hybrids were found for grain yield and its components in Western Ethiopia [9]. Significant GE interaction effects for grain yield, nutritional quality traits and milling quality indicated the presence of environment specific variability among hybrids. This indicates the importance of testing maize hybrids in diverse environments for grain yield, nutritional quality traits and milling quality. Significant GE interaction effect for grain yield of six open pollinated maize genotypes evaluated in three different locations over two seasons was reported by [13]. The phenotypic variance was higher than the genotypic variance for all traits, which suggested that the phenotypic expression of

hybrids was mainly influenced by the environment. While this may make it difficult to select for superior hybrids across environments, it has a significant implication for breeding for specific adaptation. These findings are similar to those of [20], who reported significant GE interaction effect for grain yield in maize hybrids across moisture regimes. [15] reported significant GE interaction effect for grain yield of pro-vitamin A maize hybrids in Nigeria over two cropping seasons under drought and low nitrogen environments.

High H^2 was observed for starch, fibre, milling quality, and DEFG, suggesting that the phenotypic differences were due to genotypic effects. This could further indicate that these traits could be selected with high precision based on their genotypic differences [34]. Low H^2 was seen for grain yield, protein, moisture and fat content, which indicated that the phenotypic differences of these traits were

mostly affected by the environment [35]. In contrast with the current study, high H^2 for grain yield in maize populations were found in Nigeria [36]. This could imply that the broad sense heritability values for grain yield in maize depends on genetic material and their background. Generally, most of the quantitative traits are polygenic with small additive effects, resulting in large GE interaction which results in low heritability [37]. It is challenging to improve traits with low H^2 and low percentage selection gains through selection because of high environment variance and low genetic variance.

On average grain yield ranged from 6.56 to 7.15 ton/ha, protein from 6.66 to 7.30%, starch from 66.50 to 67.24%, fibre from 2.41 to 2.50%, fat 4.02 to 4.45%, moisture from 10.92 to 11.12%, milling quality from 62.99 to 80.67% and DEFG 3.34 to 9.08% for commercial hybrids. For experimental hybrids, grain yield ranged from 5.53 to 7.25 ton/ha, protein from 6.20 to 7.36%, starch from 66.32 to 67.77%, fibre from 2.44 to 2.60%, fat from 4.03 to 4.90%, moisture from 10.78 to 11.25%, milling quality from 54.98 to 83.47% and DEFG from 3.34 to 16.80%. This indicated a wide spectrum of variability. The results also indicated high values for grain yield, nutritional quality traits and milling quality. Bojtor, *et al.* [38] evaluated 10 maize hybrids in Hungary and reported lower values for starch (62.16 to 65.05%) and oil (3.54 to 4.11%) and higher values for protein (6.30 to 7.75%) content compared to values found in this study. Two experimental hybrids (G17-Ex and G11-Ex) had the highest values for each trait in all test environments, which indicated that these hybrids may be recommended for commercial release and production. However, they should be tested for pest and disease resistance before commercial release. These results could also imply that there is a need to exploit recurrent selection in maize breeding programmes, this will ensure that the released commercial varieties are further exploited as potential parents, which is expected to increase the number of superior experimental hybrids. Moreover, these hybrids can further be evaluated for trait associations. Hybrid performance for all traits showed fluctuation between environments, which indicated that hybrids performed differently across environments. These results also showed that a hybrid that performs well in one environment will not necessarily perform well in another environment. Similar findings for 10 open pollinated varieties of maize in three different locations were found in Nepal [10]. Salinger, *et al.* [39] also reported fluctuations in maize grain yield in Veneto under the winter North Atlantic Oscillation, summer North Atlantic Oscillation, West African Monsoon and Intertropical Front.

Superior experimental hybrids G15-Ex (grain yield, fat content, and milling quality), G16-Ex (protein content and low mois-

ture content), G11-Ex (starch content) and G14-Ex (fibre content), and G2-C and G4-Ex with low DEFG were identified across environments, which suggested that they may be considered for commercial production after further evaluation for stability and adaptability in the main maize growing regions in South Africa. [40] also reported that the variation in multiple environmental trials for maize was largely attributed by the effect of the environment.

There were significant positive correlations between low DEFG with milling quality and protein with fibre and milling quality indicated that when one of the traits are selected, an improvement may also be seen in the other traits. Significant positive correlations for grain yield with protein suggested possible simultaneous improvement of these traits. Previous studies also reported positive correlation for grain yield with protein content in QPM and non-QPM hybrids [41]. Contrasting results have been reported previously, for example, strong negative correlations have been observed between grain yield and protein content [42]. The strong negative correlation of starch with grain yield and protein indicates the complexity of simultaneously improving these traits, as the improvement of one trait will have a negative influence on the other traits. The reason for this is that maize kernels contain 80% starch and 12% protein, therefore if protein is increased starch will decrease and vice versa. This might be because starch synthase enzyme activity fluctuates with protein content, resulting in changed starch content and grain size [43]. Another cause might be that the protein structure which is a ball-like structure, rather than a matrix linked by molecular acid bonding as in rye, barley, and wheat, which sticks to the starch granules, negatively altering the rheological characteristics of starch [44]. This could mean that protein and starch content should be monitored on a regular basis to identify cultivars that combine grain yield with desirable nutritional quality traits [45]. Fibre was strongly and negatively correlated with all traits, excluding protein and grain yield, which implies that an increase in fibre will have a negative effect on most of the maize nutritional quality traits whereas grain yield and protein content can be simultaneous improved in maize hybrids.

Improving maize nutritional traits such as protein content and milling quality should be considered when selecting for high yielding hybrids. This could aid in improving nutritional deficiencies. Positive correlations were observed for milling quality with almost all traits, indicating that an increase in grain yield and nutritional quality traits would result in a positive effect on the milling quality. However, milling quality was strongly and negatively correlated with fibre, which suggested that high fibre will have a negative influence in milling quality of maize kernels. These results could fur-

ther imply that high fibre causes hardness in maize kernels, which reduces the milling ability and the quality of end products such as flour and samp. Interestingly, physiochemical properties of Simiao rice with different degrees of milling showed that each milling step led to increased total starch content and amylose while decreasing dietary fibre, protein and lipids [46]. Quinoa, wheat and barley were also reported to have similar physiochemical properties as Simiao rice [47]. Even though fibre is strong negatively correlated with milling quality, both traits are important contributors to the nutritional quality of maize. Soluble fibre has been shown to lower cholesterol and reduce other health risk factors such as heart disease and stomach cancer [48]. Future studies should investigate the proportion of fibre components such as soluble vs insoluble fibre and their correlation with milling quality and other nutritional quality traits to successful breed for balance of traits in maize crop.

The PCA identified groups of hybrids that were associated with specific traits. Hybrids G1-C, G3-C, G5-C, G6-C, G8-C, G16-Ex and G17-Ex were associated with high protein and grain yield, indicating the possibility of simultaneous improvement of maize grain yield and protein content for these hybrids. These hybrids can further be used as potential varieties for commercial release and production. It is important to note that the majority of African nations and other regions of the world suffer from malnutrition and undernourishment. This might be attributable to the fact that most of these nations' daily meals are staple foods (maize, wheat, and rice). When compared to legume crops, most staple meals give a large amount of carbohydrates as a source of energy and a low amount of protein, essential minerals and vitamins. Although carbohydrates provide energy, a healthy diet should include protein, vitamins, and other minerals. As a result, effectively breeding improved maize varieties with high protein content along with nutritional quality qualities like vitamin A and Fe is required to aid in the global fight against malnutrition and undernourishment. Only two experimental hybrids (G16-Ex and G17-Ex) were associated with high grain yield and protein, further indicating a need to incorporate the commercial inbred varieties as potential parents in a breeding programme to introduce these desirable traits.

The PCA showed that hybrids G2-C, G4-Ex, G10-C, G15-Ex and G18-Ex were associated with good milling quality, fat and moisture. These hybrids are most desirable in the maize industry due to the high milling quality. However, milling quality, fat and moisture were positively correlated with DEFG for hybrids G2-C, G4-Ex, G10-C, G15-Ex and G18-Ex indicating that an improvement of these traits will be associated with high levels of defective grain. A selection index may be used to ensure the balance of grain yield

with quality traits. Milling quality or milling index is a measure of the milling ability and quality of maize kernels, with better milling quality implying more extractable and high-grade of lucrative products such as samp, maize rice, and maize grits (degermed goods) made from the corneous part of the endosperm [49]. Most of the population in SSA consumes maize in the form of samp and pap/porridge and the quality of these products depends on maize milling ability. Breeding for superior maize hybrids with high starch, protein content, nutritional quality traits like vitamin A, Fe and Zn, and as well as high milling quality is required to reduce food insecurity and hunger in SSA and other regions of the world.

The clustered heat map did not group the hybrids based on whether they were commercial or experimental hybrids. The one cluster of hybrids (G4-Ex, G5-C, G6-C, G8-C, and G11-Ex) was associated with high grain yield but also associated with lower values for important traits such as protein and milling quality. Interesting was that the cluster of hybrids (G3-C, G10-C, G12-Ex, G15-Ex, and G18-Ex) that was associated with high milling quality, was also associated with low values for protein and grain yield, indicating that the improvement of these two important maize traits will have a negative influence on the milling ability of maize. These results could indicate that there is a need for the development of a selection index in a maize breeding programme to ensure multiple trait selection and improvement of traits without a penalty to other traits.

Conclusions

The study revealed the presence of variability among maize hybrids and across environments which can be exploited for future crop improvement. Broad-sense heritability and predicted selection gain for grain yield were low, highlighting the complexity in the genetic improvement of grain yield. Broad-sense heritability was high for almost all the nutritional quality traits. Superior experimental hybrids G15-Ex (grain yield, fat content, and milling quality), G16-Ex (protein and low moisture content), G11-Ex (starch content), G14-Ex (fibre content), G2-C and G4-Ex (low DEFG) were identified, these hybrids could be released as commercial hybrids for maize production. Significant positive correlation observed between grain yield with protein and milling quality with low DEFG indicated the possibility of simultaneous trait selection and improvement in maize breeding programmes. Grain yield and protein were positively correlated and both traits were negatively correlated with starch, thus, biofortification or gene pyramiding could be considered for multiple traits improvement. Only two experimental hybrids (G16-Ex and G17-Ex) were associated with both high grain yield and protein content. Hybrids G2-C, G4-Ex, G10-C, G15-Ex and

G18-Ex were associated with good milling quality, fat, low moisture and low DEFG. The clustered heat map showed that hybrids G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex were associated with high milling quality. Maize breeding programmes should consider the development of a selection index to ensure multiple trait selection and for improving grain yield and nutritional quality traits, which will ultimately ensure the balance in trait combinations within superior cultivars. However, these results were only based on single year multi-environment trial in the eastern region of South Africa, thus these hybrids need to be evaluated in the western region and over seasons to derive a more robust conclusion on their performance and interrelationships among traits.

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Conflict of Interest

The authors have no conflict of interest to declare that are relevant to the content of this article.

Bibliography

1. Aman J, *et al.* "Correlation and path coefficient analysis of yield and yield components of quality protein maize (*Zea mays* L.) hybrid at Jimma, Western Ethiopia". *International Journal of Agronomy* 2020 (2020): 1-7.
2. Prasanna BM, *et al.* "Quality protein maize". *Current Science* 81 (2001): 1308-1319.
3. Capuano E. "The behaviour of the dietary fibre in the gastrointestinal tract determines its physiological effect". *Critical Review in Food Science and Nutrition* 57 (2017): 3543-3564.
4. Adeniyi OO and OS Ariwoola. "Comparative proximate composition of maize (*Zea mays* L.) varieties grown in south-western Nigeria". *International Annals of Science* 7 (2019): 1-5.
5. Agyeman A and M Ewool. "Genotype by environment interaction analysis of grain yield and yield components in provitamin A maize". *Journal of Crop Improvement* 3 (2021): 1-20.
6. Babic M, *et al.* "Genotype by environment interaction in maize breeding". *Genetika* 40 (2008): 303-312.
7. Bartaula S, *et al.* "Variability, heritability and genetic advance of maize (*Zea mays* L.) genotypes". *Research in Agriculture, Livestock and Fisheries* 6 (2019): 163-169.
8. Boshev D, *et al.* "Evaluation of maize hybrids grain yield stability under rainfed and irrigated conditions using GGE biplot analysis". *Bulgarian Journal of Agricultural Science* 20 (2014): 1320-1325.
9. Belay N. "Genetic variability, heritability, correlation and path coefficient analysis for grain yield and yield component in maize (*Zea mays* L.) hybrids". *Advances in Crop Science and Technology* 6 (2018): 399-405.
10. Magar BT, *et al.* "Genetic variability and trait association in maize (*Zea mays* L.) varieties for growth and yield traits". *Heliyon* 7 (2021): 1-6.
11. Nzuve F, *et al.* "Analysis of genotype by environment interaction for grain yield in maize hybrids". *Journal of Agricultural Science* 5 (2013): 75-85.
12. Tena E, *et al.* "Heritability and correlation among sugarcane (*Saccharum* spp.) Yield and some agronomic and sugar quality traits in Ethiopia". *American Journal of Plant Sciences* 7 (2016): 1453-1477.
13. Abate M. "Genotype by environment interaction and yield stability analysis of open pollinated maize varieties using AMMI model in Afar Regional State, Ethiopia". *Journal of Plant Breeding and Crop Science* 12 (2020): 8-15.
14. Badu-Apraku B and R Akinwale. "Identification of early maturing maize inbred lines based on multiple traits under drought and low N environments for hybrid development and population improvement". *Canadian Journal of Plant Science* 91 (2011): 931-942.
15. Obeng-Bio E, *et al.* "Genetic analysis of grain yield and agronomic traits of early provitamin A quality protein maize inbred lines in contrasting environments". *Journal of Agricultural Science* 157 (2019): 413-433.
16. Makore F, *et al.* "Genetic potential of new maize hybrids in single-cross hybrid combinations under low-nitrogen stress and optimal conditions". *Agronomy Journal* 12 (2022): 2205-221610.
17. Rusinamhodzi L, *et al.* "Performance of elite maize genotypes under selected sustainable intensification options in Kenya". *Field Crops Research* 249 (2020): 107738-107747.
18. Amegbor IK, *et al.* "Genetic analysis and yield assessment of maize hybrids under low and optimal nitrogen environments". *Heliyon* 8 (2022a): 1-11.

19. Ayiga-Aluba J., *et al.* "Grain yield performance and stability of quality protein maize single hybrids in mid-altitude environment in Uganda". *The Journal of Agricultural Science* 10 (2018): 55-63.
20. Singamsetti A., *et al.* "Genotype x environment and selection of maize (*Zea mays* L.) hybrids across moisture regimes". *Field Crops Research* 270 (2021): 108224-108236.
21. Ma'ali SH. "Additive main effects and multiplicative interaction analysis of maize yield trial in South Africa". *South African Journal of Plant Soil* 25 (2008): 185-193.
22. Akhtar S., *et al.* "Genotype by environment interaction in grain iron and zinc concentration and yield of maize hybrids under low nitrogen and optimal conditions". *Plants* 12 (2023): 1463-1480.
23. Pavlov J., *et al.* "Path analysis for morphological traits in maize (*Zea mays* L.)". *Genetika* 47 (2015): 295-301.
24. Yousuf M and M Saleem. "Correlation analysis of S1 families of maize for grain yield and its components". *International Journal of Agriculture and Biology* 3 (2001): 387-388.
25. Yahaya MS., *et al.* "Correlations and path-coefficient analysis for grain yield and agronomic traits of maize (*Zea mays* L.)". *Science World Journal* 16 (2021): 10-13.v
26. Rukundo P., *et al.* "Application of principal component analysis to yield and yield related traits to identify sweet potato breeding parents". *Journal of Tropical Agriculture* 95 (2015): 1-15.
27. Shengu MK. "Path coefficient analysis of early maturing maize (*Zea mays* L.) inbred lines in central rift valley of Ethiopia". *Plant Journal* 5 (2017): 47-50.
28. Tadesse T and T Leta. "Association and path coefficient analysis among grain yield and related traits in Ethiopian maize (*Zea mays* L.) inbred lines". *African Journal of Plant Science* 13 (2019): 264-272.
29. VSN International GenStat for Windows 19th Edition. VSN International, Hemel Hempstead, UK (2020).
30. SAS Institute. SAS/STAT user's guide, version 9.2. Carry (NC): SAS Institute. (2019).
31. Alam MA., *et al.* "Genetic variation and genotype by environment interaction for agronomic traits in maize (*Zea mays* L.) hybrids". *Plants* 11 (2022): 1-16.
32. Addinsoft. "The leading data analysis and statistical solution for Microsoft excel. Paris France" (2022).
33. NCSS Number Cruncher Statistical Software 2020. NCSS, LLC Kaysville, Utah, USA (2022).
34. Nzuve F. "Variability and correlation studies of grain yield and related agronomic traits in maize". *Journal of Agricultural Science* 6 (2014): 166-176.
35. Tucker SL., *et al.* "Evaluating maize phenotypic variance, heritability and yield relationship at multiple biological scale across agronomically relevant environments". *Plant Cell Environment* 43 (2020): 880-906.
36. Ochigbo AE. "Genetic variability and heritability estimate of maize endosperms using full-sib recurrent selection scheme". *Direct Research Journal of Agriculture and Food Science* 9 (2021): 230-235.
37. Hill WG. "Understanding and using quantitative variation". *Philos Trans Royal Soc* 365 (2010): 73-85.
38. Bojtor C., *et al.* "Stability and adaptability of maize hybrids for precision crop production in a long-term field experiment in Hungary". *Agronomy Journal* 11 (2021): 2167-2180.
39. Salinger MJ., *et al.* "Linking maize yields in Veneto Italy, to large-scale atmospheric variability, circulation regimes and weather types". *The Journal of Agricultural Science* 160 (2022): 423-439.
40. Badu-Apraku B and M Oyekunle. "Genetic analysis of grain yield and other traits of extra-early yellow maize inbreds and hybrid performance under contrasting environments". *Field Crops Research* 129 (2012): 99-110.
41. Amegbor IK., *et al.* "Identifying quality protein maize inbred lines for improved nutritional value of maize in southern Africa". *Foods* 11 (2022b): 898-907.
42. Kumar A., *et al.* "Diversity among maize landraces in North West Himalayan region of India assessed by agro-morphological and quality traits". *Indian Journal of Genetics and Plant Breeding* 75 (2015): 188-195.
43. Yu W., *et al.* "Relationships between protein content, starch molecular structure and grain size in barley". *Carbohydrate Polymers* 155 (2017): 271-279.
44. Shrestha AK., *et al.* "Molecular, mesoscopic and microscopic structure evolution during amylase digestion of extruded maize and high amylose maize structures". *Carbohydrate Polymers* 118 (2015): 112-114.
45. Ertiro BT., *et al.* "Relationship between grain yield and quality traits under optimum and low-nitrogen stress environment in tropical maize". *Agronomy Journal* 12 (2022): 438-452.

46. Wang Z., *et al.* "Effects of the degree of milling on the physicochemical properties, pasting properties and *in vitro* digestibility of Simiao rice". *Grain and Oil Science and Technology* 4 (2021): 45-53.
47. Föeste M., *et al.* "Isolation of quinoa protein by milling fraction and solvent extraction". *Food and Bioproducts Processing Journal* 96 (2015): 20-26.
48. Soliman GA. "Dietary fibre, atherosclerosis, and cardiovascular disease". *Nutrients* 11 (2019): 1155-1165.
49. Fox G and M Manley. "Hardness methods for testing maize kernels". *Journal of Agricultural and Food Chemistry* 57 (2009): 5647-5657.