



# Effects of genotype by environment interaction on agronomic and functional flour properties among cassava genotypes targeted for industrial use

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## ABSTRACT

The study was carried out to evaluate the genotype by environment (G × E) interaction on physicochemical and functional properties of ten (10) cassava advanced genotypes and improved varieties. The genotypes and varieties were collected from a multi-location trial (Uniform yield) of the IITA breeding program at four research stations in Malawi. Based on the results, G × E interaction was highly significant ( $P \leq 0.001$ ) in explaining the variance of the physicochemical parameters and functional properties. Thus, G × E interaction highly influenced starch and amylopectin contents, swelling power, and water binding capacity. Additive main effect and multiplicative interaction (AMMI) analysis identified I010040, MM06/0045 and TMSL110080 genotypes and Mbundumali, Mpale and Sagonja varieties as the most stable with high yield performance hence recommended for cultivation in a wide range of environments for the production of high quality cassava flour (HQCF) and starch for various industrial applications such as the production of ethanol, biofuels, starch and glucose syrup in chemical industries; thickeners, stabilizers, and texture modifiers in food, bakery and confectionery industries.

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## 1. Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy tropical root crop that is the staple food of an estimated 800 million people worldwide (Matchaya and Nhlengethwa, 2014; Nassar and Ortiz, 2006). Cassava is grown almost exclusively by low-income, smallholder farmers, as it is one of the few staple crops that can be produced efficiently on a small scale, without the need for mechanisation or purchased inputs, and in marginal areas with poor soils and unpredictable rainfall (Nilusha et al., 2021). On the other hand, high quality cassava flour (HQCF) and cassava starch can be used for various industrial applications such as the production of ethanol and biofuels, starch and glucose syrup, and sweeteners in chemical industries; thickeners, stabilizers, and texture modifiers in food, bakery and confectionery industries; binders and adhesives in paper making and plywood industries; and fillers as well as stiffeners in textile and packaging industries (Chitedze et al., 2012; Chimphepo et al., 2021a,b). However, the recommendation of preferred cassava genotypes and varieties for such industrial applications is highly based on their functional properties, which

are influenced by physicochemical parameters. As such, cassava genotypes and varieties with high yield and desirable physicochemical and functional properties will be identified for targeted industrial uses, positively influencing the commercialization of the cassava roots for industrial applications.

The physicochemical parameters and functional properties of flours from advanced cassava genotypes and improved cassava varieties for industrial applications were evaluated by Chimphepo et al. (2021a,b). However, there is a need to identify further cassava genotypes and varieties with high yield and stability performance (with desirable physicochemical and functional properties) that can survive in a diverse range of environments to produce HQCF and starch for targeted industrial uses. Since Malawi has diverse agro-ecological zones, in terms of edaphic and climatic factors, the genotype by environment interaction (G × E) effect is inevitable (Benesi et al., 2008) and, therefore, complicates the recommendation of genotypes based on yield, physicochemical parameters and functional properties alone (Akinwale et al., 2011; Hugh and Gauch, 2013). In this context, the study was undertaken to evaluate the G × E interaction on cassava qualities desirable for industrial applications: root dry matter content, bulk density, starch and amylopectin content, water binding capacity, oil absorption capacity, swelling power and solubility of cassava flour (Chimphepo et al.,

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2021a,b). As such, the results of this study will contribute to efforts to fast-tracking the suitable cassava genotypes and varieties with high yield and stable performance for various industrial applications.

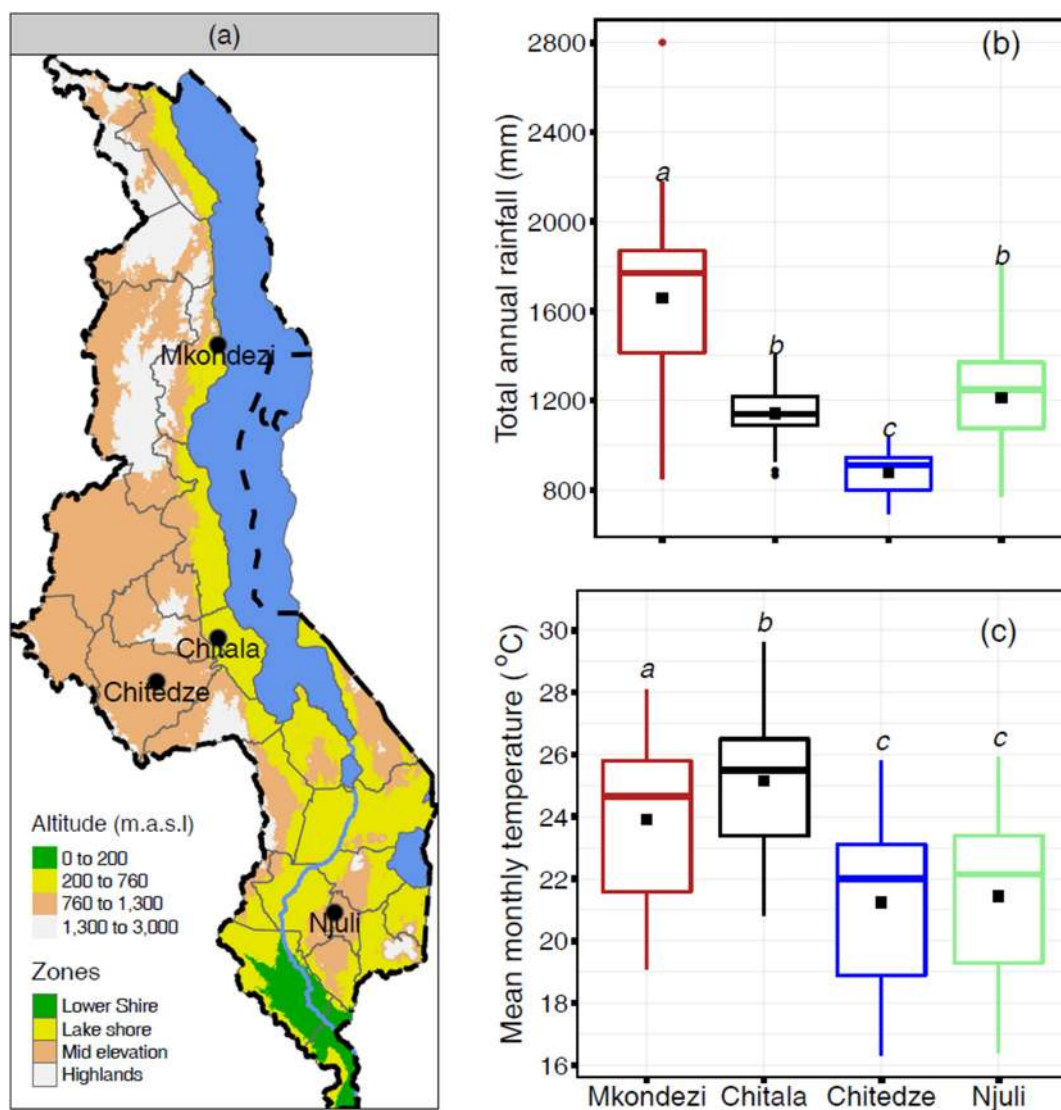
## 2. Materials and methods

### 2.1. Study sites

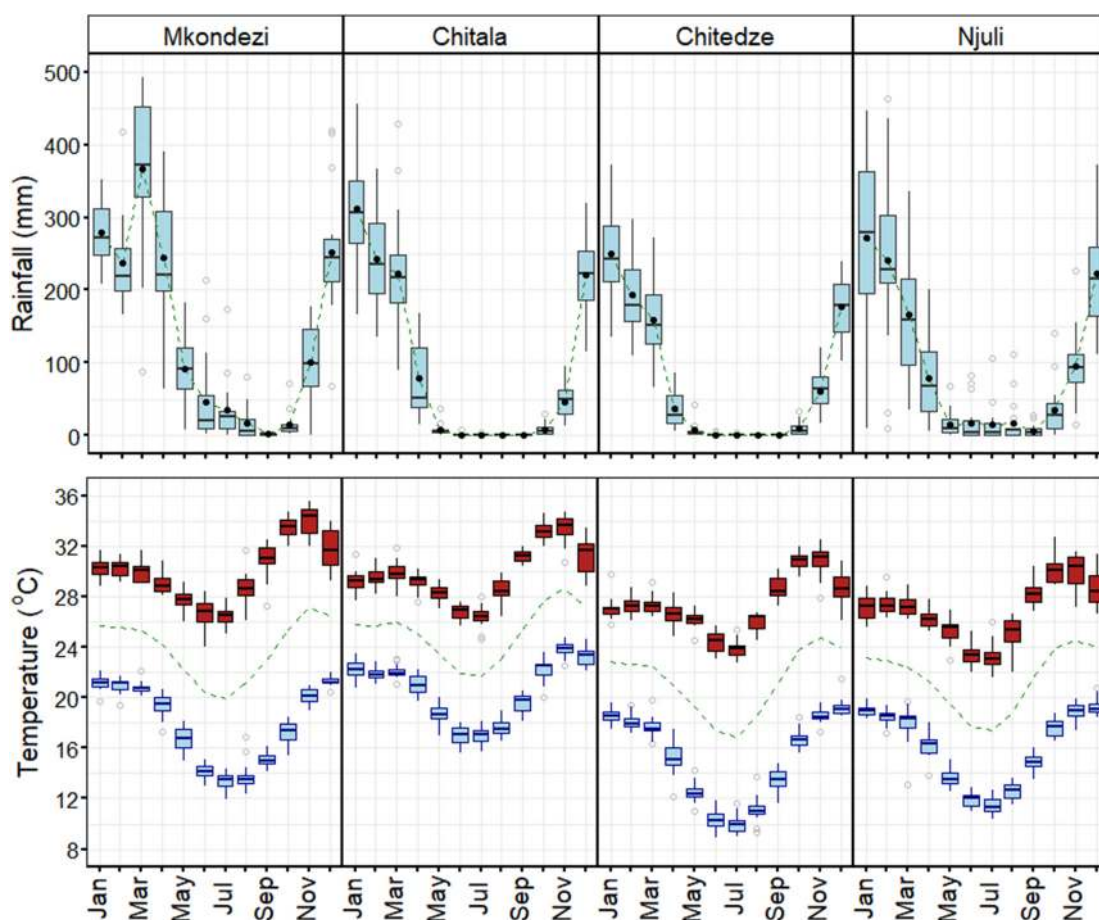
Cassava samples were collected from the IITA uniform yield trial planted in the 2016/17 season at three Agricultural Research Stations of Chitala, Chitedze and Mkondezi and Njuli farm in Malawi. The research sites are located in different agro-ecological zones representing heterogeneity in terms of soil type, elevation, and meteorological conditions (Figs. 1, 2 and Table 1). In Malawi, the agro-ecological zones are categorised, mainly according to elevation (Fig. 1a), as Lower Shire valley (altitude below 200 m.a.s.l), Lake shore, middle and upper Shire valley (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l) (Matchaya and Nhlengethwa, 2014). The spatial variation of climate variables (temperature, humidity and rainfall) depends on elevation, and therefore, the agro-ecological zones

represent the spatial climatic zonation of the country (Ngongondo et al., 2011).

Chitala and Mkondezi research stations are located in the lake shore, upper and middle Shire valley agro-ecological zone, whereas Chitedze and Njuli are situated in the mid-elevation agro-ecological zone (Fig. 1a). All locations have a tropical wet and dry “savanna” climate (Ngongondo et al., 2011), characterized by a distinct rainy season between November and April and hot and cool from October to December and May to July, respectively (Fig. 2). In general, the lake shore, upper and middle Shire valley agro-ecological zone (Mkondezi and Chitala stations) is characterized by higher mean monthly temperatures than the mid-elevation zone (Fig. 2). Monthly mean temperatures at Mkondezi, Chitala, Chitedze and Njuli fall in the range 19–28 °C, 20.8–29.6 °C, 16–25.8 °C and 16–25.9 °C, respectively (Figs. 1c and 2). In terms of rainfall, the area of Mkondezi research station receives higher total annual rainfall of over 1500 mm per year than the other stations (Fig. 1b), which peaks in March (Fig. 2). For the 2016–2017 growing season, total annual rainfall of 1021 mm, 982 mm, 965 mm and 940 mm were received at Mkondezi, Chitala, Chitedze and Njuli stations, respectively. For the same period, the average monthly



**Fig. 1.** Location, temperature, and rainfall patterns of the research sites. (a) Map showing location of the research sites in Malawi's agro-ecological zones: Lower Shire valley (altitude below 200 m.a.s.l), Lake Shore, middle and upper Shire (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l). (b) Monthly average rainfall and (c) monthly average temperatures at each research station for the period 1995–2012 indicating the climate characteristics of the areas in which the stations are located. Data from Malawi Department of Climate Change and Meteorological Services (DCCMS).



**Fig. 2.** Barplots showing the monthly distribution of rainfall (upper panel) and maximum, minimum and mean temperature (lower panel) at each research station for the period 1995–2012, indicating the climate characteristics of the areas in which the stations are located. The green dashed lines represent average monthly rainfall (upper panel) and temperature (lower panel). Data from Malawi Department of Climate Change and Meteorological Services (DCCMS).

temperatures were in the range of 22–28 °C, 22–29 °C, 17–24 °C and 17–24 °C at Mkondezi, Chitala, Chitedze and Njuli, respectively.

At Mkondezi, the dominant soils are strongly acidic sandy loam to sandy clay, with variable low to medium nutrient levels because of leaching. They are low in nitrogen, phosphorous and cation exchange capacity (CEC). Exchangeable potassium is marginally adequate (Table 1), whereas calcium and magnesium are favourable only at selected sites (Bationo et al., 2012). Chitala and Chitedze soils are slightly acidic sandy clay and sandy clay loam with lower phosphorus (P) levels (Table 1). Chitedze soils have relatively higher levels of potassium (K). The soil texture varies from sand clay to sand clay loam (Table 1), and these differences may cause significant moisture and nutrient holding capacity variations.

## 2.2. Sample collection

The cassava samples comprised of Mpale, Mbundumali, Sauti and Sagonja varieties (hereafter referred to as genotypes), and I010040,

I010085, I020452, TMSL110080, TMEB419 and MM06/0045 genotypes which were planted on 22 December 2016 in a randomized complete block design with four replications. Four replicates of the sample were harvested at 12 months later from only two middle rows and then processed within 24 h of harvesting. The cassava roots were washed, peeled and sliced into 20 mm (thickness) pieces oven-dried at 60 °C for 48 h (Kehinde et al., 2014). The dried chips were ground into flour using a laboratory mortar and pestle, sieved through a 0.25 mm metal mesh to produce a consistent 0.25 mm particle size and then packaged in polythene bags and stored awaiting analysis.

## 2.3. Determination of physicochemical and chemical parameters of cassava flours

### 2.3.1. Determination of root dry matter contents of cassava

Fresh root dry matter content was determined using the oven method as described by Chimphepo et al. (2021a,b). 10 g of fresh cassava sample, weighed in pre-weighed dishes, dried in an oven at

**Table 1**  
Edaphic description of trial sites.

	Mkondezi	Chitedze	Chitala	Njuli
pH (H <sub>2</sub> O)	4.0–5.6	5.2–5.8	5.6–6.1	5.73–6.41
Organic carbon(g/100 g)	2–5	2.39	1.07	2.88–3.47
Nitrogen (g/100 g)	0.04–0.2	0.16	0.04–0.07	0.25–0.29
Phosphorus (µg/g)	20–50	15.57–24.81	10.4–19.39	59.31–83.21
Potassium (cmol/kg)	0.08–0.14	0.31–0.40	0.23–0.51	0.18–0.26
Soil texture	Sandy clay to clay	Sandy clay loam	Sandy clay to sandy clay loam	Sandy clay loam
References	Benesi et al., 2008	Benesi et al., 2008; Bationo et al., 2012	Benesi et al., 2008; Bationo et al., 2012	

110 °C overnight, cooled the following morning in a desiccator for 2 h and weighed again.

### 2.3.2. Determination of bulk density of cassava flours

Bulk density was estimated following the method used by Iwe et al. (2017). The flour sample (10 g) was put into a 25 mL volumetric cylinder. The lower surface of the cylinder was tapped several times on the laboratory bench until there was no more diminution of the sample level. The sample weight was then determined, and bulk density was expressed as the weight/volume of the sample (g/mL).

### 2.3.3. Determination of starch, amylose and amylopectin contents of cassava flours

Total starch (anthrone reagent) content and amylose (iodine reagent) content were determined by UV/VIS spectrophotometry (Oladayo et al., 2016). For starch analysis, 100 mg of the flour samples (cassava and HQCF) were weighed into 50 mL centrifuge tubes and homogenized with 30 mL of hot 80 % ethanol to remove sugars and then centrifuged for 10 min (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E) and residue retained. The residue was washed repeatedly with hot 80 % ethanol until the washings did not give the colour with anthrone reagent. The residue was dried well over a water bath. 5.0 mL of water and 6.5 mL of 52 % perchloric acid were added to the dried residue, and then total starch was extracted at 0 °C for 20 min. After 20 min of total starch extraction, the sample was centrifuged for 5 min, and the supernatant was saved. The supernatant was made up to 100 mL by distilled water. Then 0.1 mL of the supernatant was pipetted into a boiling tube using a micropipette and made up to 1.0 mL with distilled water.

Thus, to determine total starch content, calibration curves were derived using D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA), where the stock solution was prepared by dissolving 100 mg of glucose in 100 mL of distilled water, and then working standards of glucose were prepared (by diluting 10 mL of stock solution into 100 mL flask to its mark) as 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard of D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA) which were also made to the mark of 1 mL volume and "0" served as a blank. Then 4 mL of anthrone (400 mg dissolved in 200 mL of ice cold 98 % sulphuric acid) were added to the samples, as well as to the standard solutions of glucose and boiled (100 °C) for 8 min on a water bath, after cooling, standards and samples were read on UV/VIS Spectrophotometer (S/N: 20-1901-0351; Model: T90+; PG instruments Ltd) at 630 nm. Glucose concentration in the sample was estimated using a calibration curve and Eq. (1).

$$\text{Glucose concentration} = \frac{\text{Absorbance}}{m \times 0.01\text{mL}} \times 100\text{mg} \quad (1)$$

where m = Slope of calibration curve

Then starch content was calculated by multiplying the value of glucose content estimated from above by a factor of 0.9.

Amylose content was determined by UV/VIS spectrophotometry (Oladayo et al., 2016), 100 mg of cassava flour sample was added to 1 mL of 99.9 % ethanol, and then 10 mL of 1 N NaOH (4 g of NaOH pellets was dissolved in 100 mL of distilled water) was added and left overnight. Then the volume was increased to 100 mL using distilled water. A 2.5 mL of the extract was taken, and 20 mL of distilled water was added, followed by 3 drops of phenolphthalein. Then 0.1 N Hydrochloric acid was added drop by drop until the pink colour just disappeared. Then 1.0 mL of iodine reagent (1.0 g of Iodine and 10 g of KI dissolved in distilled water and made up to the mark of 500 mL volumetric flask) was added and blue black colour developed and the volume was increased to 50 mL using distilled water.

Calibration curves were derived using pure amylose from potato (A0512; Sigma–Aldrich, St. Louis, MO, USA), prepared (100 mg amylose

was dissolved in 10 mL of 1 N NaOH and the volume increased to 100 mL using distilled water) as 0.2, 0.4, 0.6, 0.8 and 1 mL, and the colour was developed as in the case of the sample. For a blank, 1.0 mL of iodine reagent was diluted to 50 mL with distilled water. Hence the colour developed for samples and amylose standards was read on UV/VIS Spectrophotometer (S/N: 20-1901-0351; Model: T90+; PG instruments Ltd) at 590 nm. Eq. (2) was used to calculate the amount of amylose in cassava flours.

Absorbance that corresponds to 2.5 mL of the test solution

$$= x \text{ mg amylose } 100 \text{ mL contains} = \frac{x}{2.5} \times 100 \text{ mg} = \% \text{Amylose} \quad (2)$$

where x = Glucose concentration

The amylopectin content of a flour sample was calculated as a difference between total starch content and amylose content of the flour sample (Oladayo et al., 2016).

## 2.4. Determination of functional properties of cassava flours

### 2.4.1. Determination of swelling power and water solubility of cassava flours

Swelling power and water solubility were determined using methods described by Kusumayanti et al. (2014). To determine the swelling power of cassava flours, a 0.1 g flour sample was mixed with 10 mL distilled water and heated at 90 °C for 1 h, with constant mixing. Then, the suspension was cooled rapidly, equilibrated at 25 °C and centrifuged for 30 min at 1600 rpm (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E), and then the sediments were weighed. For solubility, a 0.5 g flour sample was heated in 10 mL distilled water at 60 °C (in a water bath) for 30 min, without mixing. The sample was centrifuged at 1600 rpm for 10 min rpm (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E). The supernatant (5 mL) was separated, dried and weighed. The flour's swelling power and water solubility were calculated using Eqs. (3) and (4), respectively.

$$\text{Swelling power (g/100g)} = \frac{\text{Weight of the sediments}}{\text{Weight of initial flour}} \times 100 \quad (3)$$

$$\text{Solubility (g/100g)} = \frac{\text{Dried supernatant weight}}{\text{Weight of initial flour}} \times 2 \times 100 \quad (4)$$

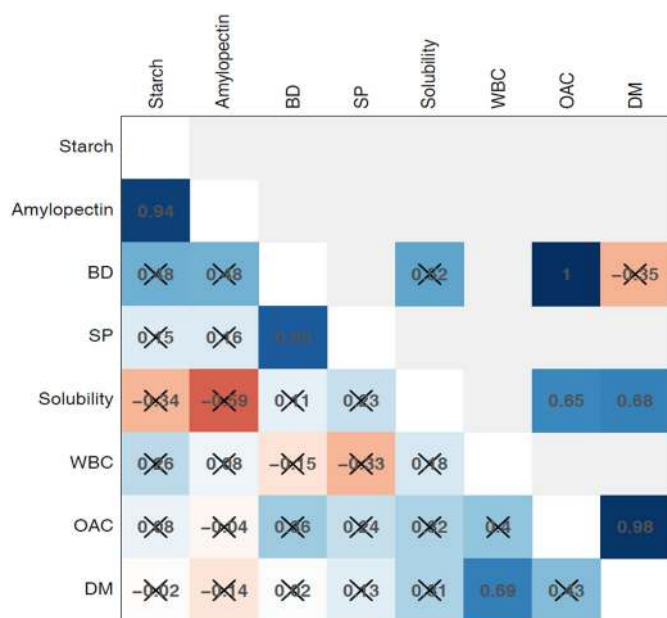
### 2.4.2. Determination of water binding and oil absorption capacities of cassava flours

Water binding capacity and oil absorption capacity were determined according to methods described by Agyepong and Barimah (2018) and Iwe et al. (2017), respectively. 2.0 g of the flour sample was dissolved in 40 mL of water in a centrifuge tube for water binding capacity. The suspension was agitated for 1 h at room temperature on a shaker and centrifuged for 10 min at 2200 rpm. The free water was decanted from the pellet, drained for 10 min, and pellet weighed. For oil absorption capacity, 1 g flour sample was mixed with 10 mL soybean oil (Specific gravity: 0.9092) and allowed to stand at ambient temperature (30 ± 2 °C) for 30 min centrifuged for 30 min at 300 rpm. Water and oil absorption capacities were determined using Eq. (5).

$$\text{Water (Oil)Absorption Capacity (g/100 g)} = \frac{\text{Weight of absorbed water (Oil)}}{\text{Weight of initial flour}} \times 100 \quad (5)$$

## 2.5. Data analysis

Software R version 4.1.0 (A language and environment for statistical computing) was used for the analysis of variance (ANOVA) and all other statistical analyses such as phenotypic and genotypic correlations across



**Fig. 3.** Phenotypic (below diagonal) and genotypic (above diagonal) correlation matrices for dry matter content, bulk density, starch and amylopectin content and functional properties of cassava flours.  $P \leq 0.05$ , insignificant correlations are crossed out. Dark blue:  $r^2 = 1$ ; dark red:  $r^2 = -1$ . Positive correlations are displayed in blue and negative correlations in red colour. Colour intensity is proportional to the correlation coefficients. WBC = Water Binding Capacity; OAC = Oil Absorption Capacity; DM = Dry Matter Content (Fresh weight basis); BD = Bulk density, SP = Swelling power.

all trials (multi-environment trial analysis, META R Ver 5.0) (Alvarado et al., 2020). ANOVA was performed on physicochemical and chemical parameters (root dry matter content, bulk density, starch and amylopectin content of flour) and functional properties of cassava flour (swelling power, water binding capacity, oil absorption capacity and solubility) of each of the individual trials. After that, an additive main effect and multiplicative interaction (AMMI) analysis was performed to identify the most stable genotypes and location (Akinwale et al., 2011; Hugh and Gauch, 2013) and allocate all unstable genotypes to the most suitable location (Hugh and Gauch, 2013). The bi-plots of IPCA1 vs mean response variable were produced using the ggplot2 package to visually evaluate the performance and stability of genotypes (and

varieties) and environments. In addition, stability tests such as Shukla's stability variance and Kang's yield-stability statistics were calculated to identify most stable genotypes by simultaneous selection for stability and yield performance variable. The selected genotypes were then ranked according to AMMI yield stability index (YSI) (Sabaghnia et al., 2008) to identify most stable cassava genotypes and varieties with high yield according AMMI.

### 3. Results and discussion

#### 3.1. Correlations and variations of fresh root dry matter content and cassava flour quality parameters with genotype and location

Data used in this study are deposited in a Mendeley public repository (Chimphepo et al., 2021b). Phenotypic correlations of physicochemical parameters and functional properties revealed high significant ( $P \leq 0.05$ ) positive correlations for starch content with amylopectin content, bulk density with swelling power (SP), and dry matter content with water binding capacity (WBC) (Fig. 3). On the other hand, genotypic correlations showed high significant ( $P \leq 0.05$ ) positive correlations for bulk density with oil absorption capacity (OAC) and dry matter content with solubility and OAC (Fig. 3). Both correlations showed a relationship between physical parameters (bulk density and dry matter content) and functional properties (OAC, WBC, SP and solubility). Chimphepo et al. (2021a,b) found that starch and amylopectin content were the major determinants of variability in cassava flours' functional properties, such as water and oil absorption capacities, solubility, and swelling power. However, the study further looked at the expression of the crop in terms of functional properties, where the phenotypic correlation may be more appropriate (Benesi et al., 2008).

Variations for bulk density, root dry matter content, chemical parameters such as starch and amylopectin contents, and functional properties, namely; swelling power, solubility, water binding capacity and oil absorbance capacity, were highly significant ( $P \leq 0.001$ ) for the main effects of genotype (G) and environment (E) as well as  $G \times E$  interaction (Table 2). The results further show that the environment highly influenced fresh root dry matter content, bulk density, and solubility, and contributed 49.82 %, 85.10 %, and 85.10 %, respectively, to the total sum of squares (Table 2). Furthermore, fresh root dry matter content strongly depends on the edaphic-climatic and agronomic conditions (Benesi et al., 2008). On the other hand, genotype and environment interaction played a major role in influencing starch content,

**Table 2**  
ANOVA for physicochemical parameters (bulk density, dry matter content), chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity (WBC) and oil absorbance capacity (OAC)) for cassava flours.

		Total	Location (L)	Rep (L)	Genotype (G)	G × L	Residual
Source of variation	DF	127	3	3	9	23	89
Starch content (g/100 g)	Sum of squares (SS)	3772.88	1206.13**	15.72**	620.1***	1885.17***	45.76
	Contribution to total SS (%)		31.97	0.42	16.44	49.97	1.21
Dry matter content (g/100 g)	Sum of squares (SS)	8135.60	4053.40***	150.50*	2176.70***	1147.60***	607.4
	Contribution to total SS (%)		49.82	1.85	26.76	14.11	7.47
Bulk density (g/mL)	Sum of squares (SS)	0.544	0.463***	0.006*	0.019***	0.030***	0.026
	Contribution to total SS (%)		85.10	1.16	3.54	5.47	4.73
Amylopectin (g/100 g)	Sum of squares (SS)	4154.01	1615.79***	10.25	595.55***	1872.01***	60.41
	Contribution to total SS (%)		38.90	0.25	14.34	45.07	1.45
Swelling power (g/100 g)	Sum of squares (SS)	156.60	11.76*	6.43	14.47***	91.47***	32.46
	Contribution to total SS (%)		7.51	4.11	9.24	58.42	20.73
Solubility (g/100 g)	Sum of squares (SS)	0.54	0.46***	0.006*	0.019***	0.030***	0.026
	Contribution to total SS (%)		85.10	1.16	3.54	5.47	4.73
WBC (g/100 g)	Sum of squares (SS)	95,302	30486***	1291*	10454***	47810***	5261
	Contribution to total SS (%)		31.99	1.35	10.97	50.17	5.52
OAC (g/100 g)	Sum of squares (SS)	34,507.20	10523***	1576**	7657***	106370***	4115
	Contribution to total SS (%)		30.49	4.57	22.19	30.82	11.93

ns, not significant. WBC = Water Binding Capacity; OAC = Oil Absorption Capacity; DF = Degrees of freedom; SS = Sum of squares; ANOVA = Analysis of variance.

\*\*\* Significant at  $P \leq 0.001$ .

\*\* Significant at  $P \leq 0.01$ .

\* Significant at  $P \leq 0.05$ .

amylopectin content, swelling power, and WBC, with contributions of 49.97 %, 45.07 %, 58.42 %, and 50.17 %, respectively, of the total sum of squares (Table 2). OAC was influenced by genotype, location, and interaction with contributions of 22.19 %, 30.49 %, and 30.82 %, respectively, of the total sum of squares (Table 2).

In general, the results from this study were in agreement with similar studies that reported variations in starch and dry matter content across genotypes (or varieties) and locations (Benesi et al., 2004; Sriroth et al., 2000). Variations in dry matter content, starch and amylopectin content and bulk density were highly significant ( $P \leq 0.05$ ) for genotypes and locations (Fig. 4). Mkondezi was the best site for dry matter content, followed by Njuli, with TMEB419, Mpale and Mbundumali as the highest yielding varieties and genotypes (Fig. 4). Chitala was the best site for bulk density, starch and amylopectin content (Fig. 4). Cassava genotypes and varieties that gave the highest starch content, amylopectin content and bulk density at Chitedze were Sagonja, I020452, TMSL110080, TMEB419 and I010040 for bulk density, I010040, TMSL110080 and I010085 for starch content and amylopectin

content (Fig. 4). In addition, most flour properties obtained in this work were comparable with previous studies registering high starch ranging from 72.39 g/100 g to 84.15 g/100 g and high amylopectin content ranging from 64.49 g/100 g to 74.50 g/100 g, high bulk density ranging from 0.65 g/mL to 0.69 g/mL (Agyepong and Barimah, 2018; Benesi et al., 2004; Sriroth et al., 2000).

In terms of functional properties, Mkondezi was the best site for WBC, whereas Njuli was the least performing site. Chitala was the best site for oil absorption capacity, swelling power and solubility (Fig. 5). Cassava genotypes and varieties that gave high values at Chitedze were Mbundumali, Mpale, Sagonja, TMSL110080 and I010040 for OAC; Mbundumali, MM06/0045, I020452, TMSL110080, I010040 and I010085 for swelling power; and starch content while TMEB419, I010085, Mbundumali, MM06/0045, I010040 were for solubility (Fig. 5).

The results show that the best sites for bulk density, dry matter content, chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity and oil absorbance capacity) were Chitala and Mkondezi sites

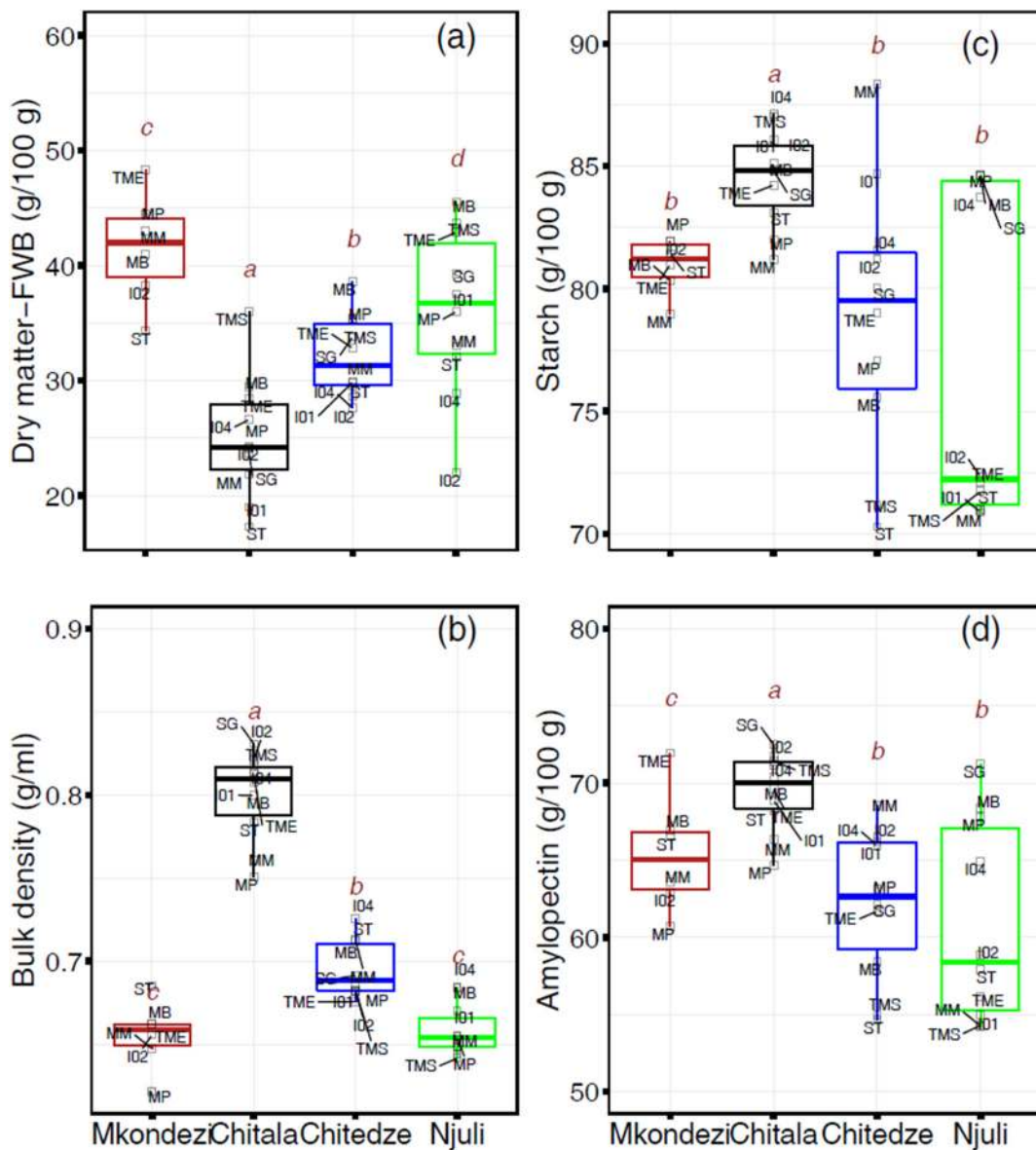


Fig. 4. Dry matter content, bulk density, starch, and amylopectin content (of cassava flours) of cassava genotypes and varieties for different trial sites. Research sites with the same letter have insignificant differences ( $P = 0.05$ ). Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.

located in the Lake Shore agro-ecological zone. Genotypes TMEB419, MM06/0045, I020452 and varieties of Sauti, Mbundumali and Mpale had high dry matter content in Mkondezi. Dry matter content is closely related to soil moisture content during 6–18 months of plant growth, which is a function of the amount and distribution of rainfall and soil properties (Byju and Suja, 2020). Most of the sites have sandy loam and sandy clay loam soils (Table 1), which are the most preferred soil types for tuberous root development (Bationo et al., 2012) and also provide better soil nutrient retention (Byju and Suja, 2020). On the other hand, all these analysed functional properties gave high values consistent with what other studies found (Agyepong and Barimah, 2018; Benesi et al., 2004; Iwe et al., 2017).

Generally, the Mkondezi research station receives higher (>1500 mm per year) (Fig. 1) and well-distributed rainfall than the other stations (Benesi et al., 2008). For optimum growth and production, cassava requires an annual rainfall of >1000 mm (El-Sharkawy and Cadavid, 2002). However, it can survive in a wide variation of rainfall conditions ranging from <600 mm in semi-arid tropics to >1600 mm in subhumid/humid tropics (Allem, 2002). High

temperatures are also known to accelerate the growth and formation of tuberous roots of cassava (Nassar and Ortiz, 2006), and cassava is adapted to tropical semi-arid conditions. The Lake Shore agro-ecological zone has a relatively higher annual temperature than the mid-elevation agro-ecological zone. In Malawi, cassava is mainly grown along with the lake shore areas of the central and northern regions (Benesi et al., 2008). Optimum annual mean temperatures for growth and tuberous root production range from 25 to 30 °C for cultivars adapted to cool climates and 30–36 °C for cultivars that come up well in hot-climate (El-Sharkawy, 2006). The higher dry matter content and water binding capacity at the Mkondezi site correspond to higher rainfall and soil organic matter content (Howeler, 2002) than the other sites. Potassium content in soil influences bulk density, starch and amylopectin content, solubility, swelling power and oil absorption capacity (Benesi et al., 2008). Table 1 confirms what Benesi et al. (2008) found that Chitala has high potassium content. Chitedze also shows higher soil potassium content (Benesi et al., 2008; Table 1), giving high bulk density, starch and amylopectin content, solubility, swelling power and oil absorption capacity (Figs. 4 & 5). The critical level of

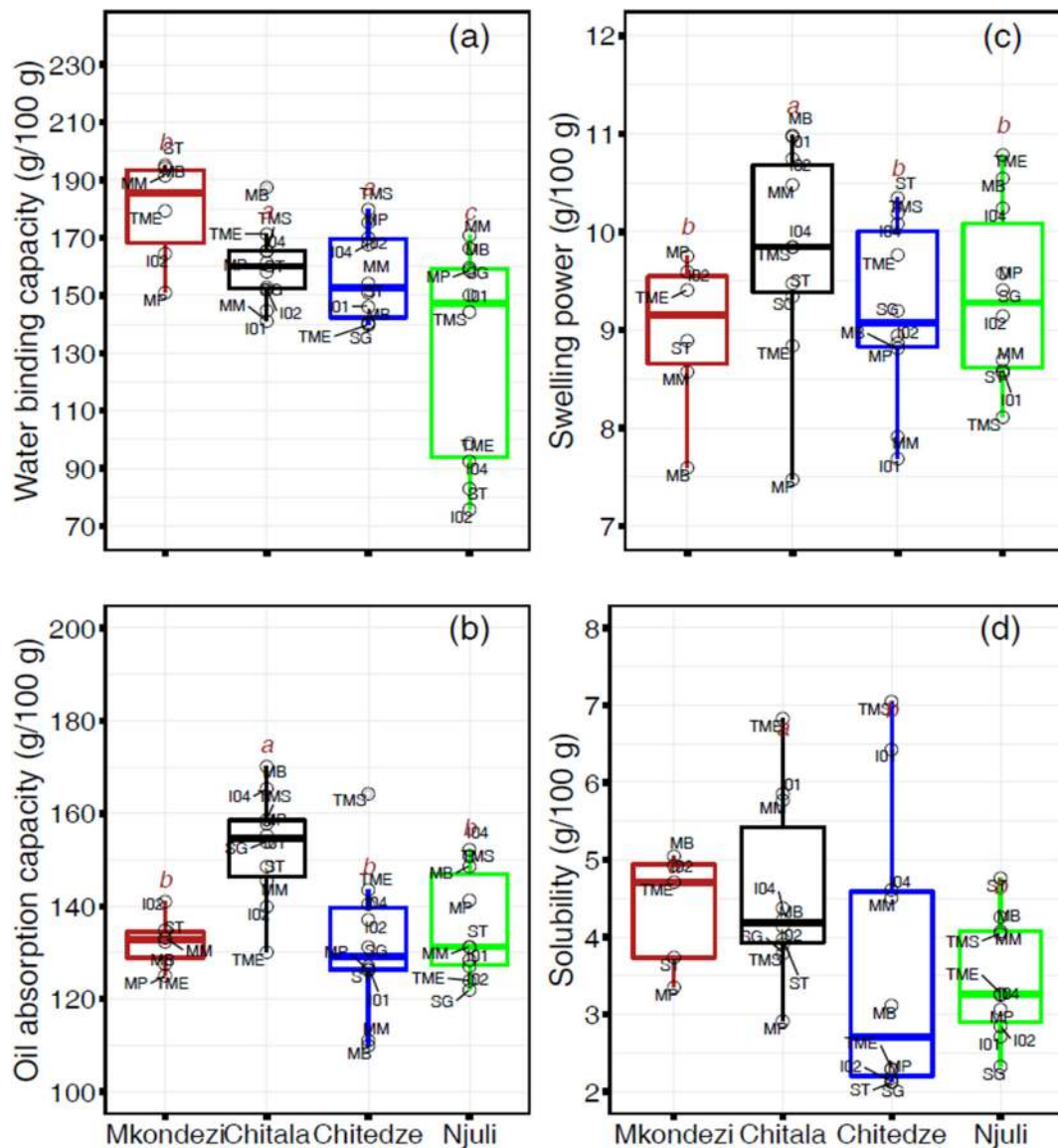


Fig. 5. Functional properties (Water binding capacity, oil absorption capacity, swelling power and solubility) of cassava flours from cassava genotypes and varieties for different trial sites. Research sites with the same letter have insignificant differences ( $P = 0.05$ ). Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.

exchangeable soil potassium for cassava is reported to be in the range of 0.15–0.25 cmol/kg (Fernandes et al., 2017). Potassium is important for starch synthesis, translocation, and tuber initiation and bulking (Howeler, 2002). Therefore, potassium is associated with total starch yield, root diameter and weight, storage cell size and number, and dry matter (Chua et al., 2020; Fernandes et al., 2017).

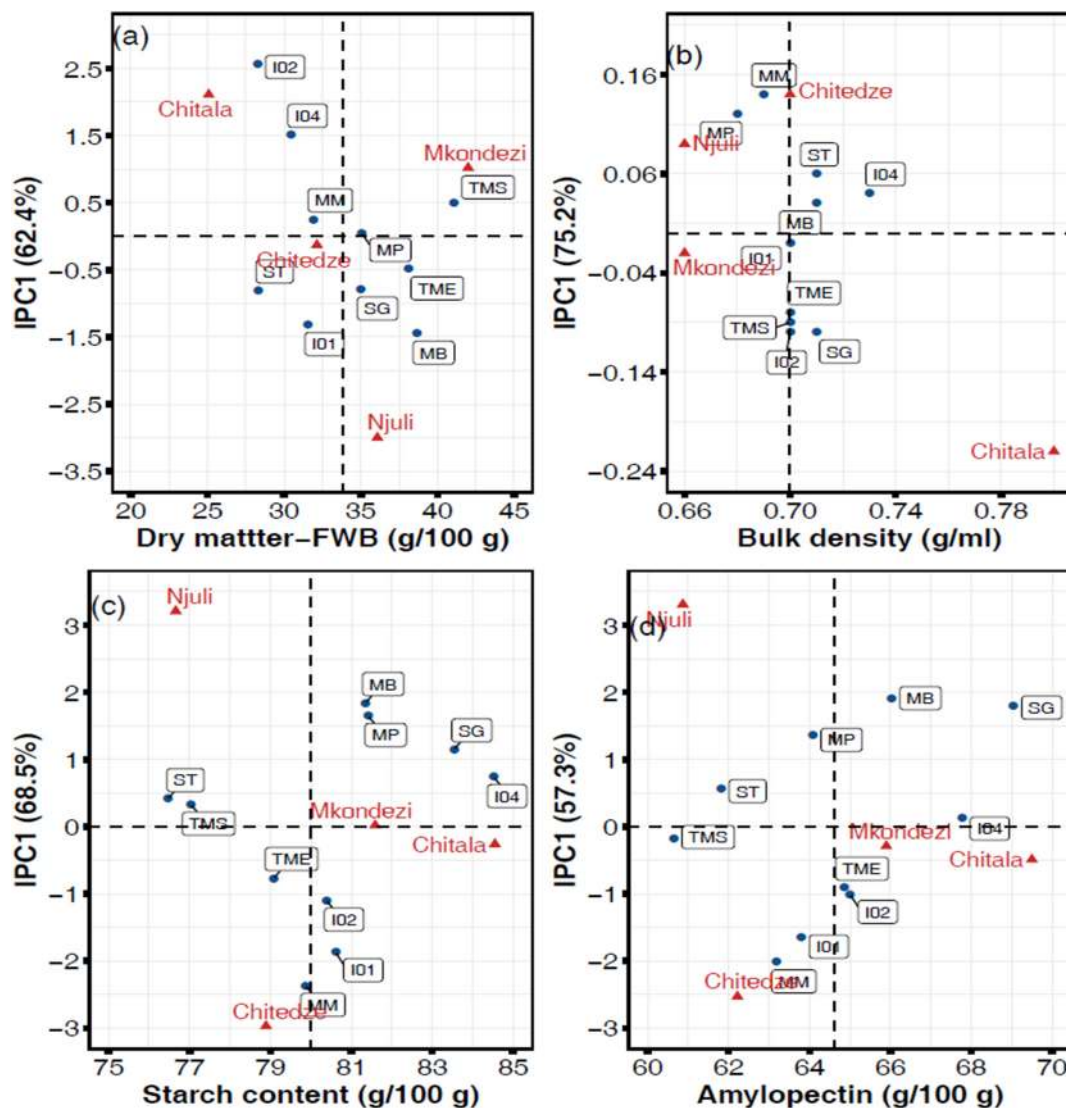
### 3.2. Stability of genotypes and varieties for fresh yield root dry matter content and cassava flour quality parameters

According to Sabaghnia et al. (2008), the stability analysis was required for the interaction of genotypes by locations (Table 2) for bulk density, dry matter content, chemical parameters (starch and amylopectin content), and functional properties (swelling power, solubility, water binding capacity and oil absorbance capacity) of the cassava flours were highly significant ( $p < 0.001$ ). AMMI analysis indicated that the first interaction principal component (IPC1) explained 62.4 % of the variation for dry matter content. IPC scores revealed that the most stable genotypes and varieties were MM06/0045, Mpale, TMEB419 and TMSL110080, with Chitedze as the most stable site. TMSL110080 had the highest fresh yield root dry matter content at

Mkondezi, and therefore, Mkondezi is the most suitable for high yield of dry matter content. The most unstable genotype was I020452 and was most suited at Chitala (Fig. 6). Chitala and Njuli were the most unstable sites for growing cassava with high dry matter content (Fig. 6).

AMMI analysis for bulk density of cassava flours from the genotypes and varieties for genotype interactions by location indicated that IPC1 explained 75.20 % of the  $G \times E$  interaction. IPC scores revealed that the most stable genotypes and varieties were I010085, I010040 and Mbundumali. Mkondezi was the most stable site, whereas Chitala was the most unstable site for bulk density (Fig. 6). The most stable genotypes for amylopectin and starch were TMSL110080, I010040, Sauti, TMEB419 and I020452. Mkondezi and Chitala were the most stable sites, with Chitala presenting a better opportunity for higher starch and amylopectin content. MM06/0045 and I010085 were the most unstable genotypes (Fig. 6).

The results showed that IPC1 explained 58.80 % and 59.80 % of the swelling power and solubility variance, respectively. TMSL 110080, I010040, I020245, Sauti and Sagonja were the most stable genotypes for swelling power (Fig. 7). Mpale was best suited at Mkondezi and Chitedze, whereas Chitala was the most unstable location for swelling power (Fig. 7). For solubility, IPC scores show that the most stable



**Fig. 6.** Biplot for AMMI IPC1 scores of the interaction term ( $G \times E$ ) against means of (a) dry matter content on fresh root weight basis, (b) starch content, (c) bulk density and (d) amylopectin content of 10 advanced genotypes and varieties, and four environments. IPC1 = Interaction principle component axis 1;  $G \times E$  = Genotype by environment; AMMI = Additive main effect and multiplicative interaction; Genotypes codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.



genotypes were I010040, Sagonja, MM06/0045, Mpale, I020452 and Mbundumali. TMEB 419 and Sauti were best suited at Chitala and Njuli, respectively. Chitedze was the most unstable location for solubility (Fig. 7).

For OAC and WBC of flours from the cassava genotypes and varieties studies, the IPC1 explained 83.1% and 71.5.80% of the variance from  $G \times E$  interaction, respectively. TMSL110080 was the most stable genotype for WBC, followed by I010040 and TMEB 419 and the varieties of Mpale and Mbundumali. Njuli was the most unstable location for water binding capacity (Fig. 7). IPC scores showed Sauti, Sagonja, I010085 and I010040 as the most stable genotypes for OAC, with Mkondezi as the most stable location. Chitala and Chitedze were the most unstable location for oil absorption capacity (Fig. 7).

Rankings of genotypes for the fresh root dry matter content and cassava flour qualities varied from one location to the other and with the particular quality parameter. The summary of the rankings of stability for genotypes was obtained by calculating the stability tests such as Shukla's stability variance (Shukla, 1972) as well as Kang's yield-stability statistics (Kang, 1993) where the most stable genotypes and varieties with high yield performance were identified in a simultaneous

selection. Then AMMI stability value (ASV) and Yield stability index (YSI) were used to rank as well as identify those selected genotypes as most stable with high yield performance (Sabaghnia et al., 2008). The most stable genotype is the one with the lowest ASV score and is ranked 1. The YSI is based on the sum of the ranking due to ASV scores and yield or performance ranking. Genotype with low YSI value is the most stable with high mean yield performance, ranking 1.

The results of the ranking and selection are shown in Fig. 8, with Mbundumali and I010040 being the most stable with high yield performance. I010040 yielded high starch and amylopectin content, bulk density, OAC, solubility and swelling power, whereas Mbundumali yielded higher fresh root dry matter content and water binding capacity (WBC) (Fig. 8). They are followed by Mpale, Sagonja, MM06/0045 and TMSL110080 genotypes (Fig. 8), with TMSL110080 having the highest yield performance of fresh root dry matter content (Fig. 8).

#### 4. Conclusion

The location influenced the fresh root dry matter content, bulk density, and solubility. In contrast,  $G \times E$  interaction influenced the starch

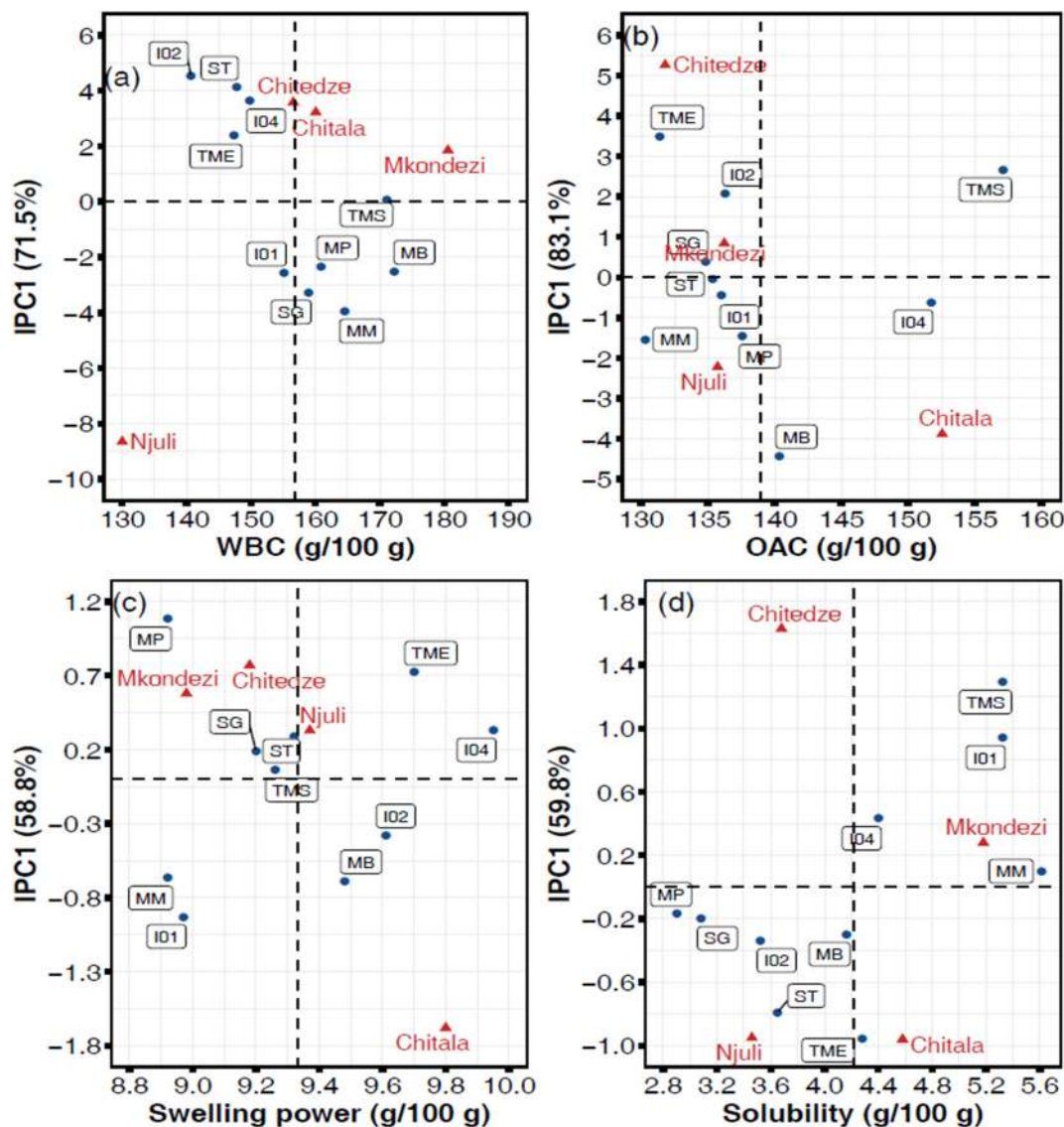
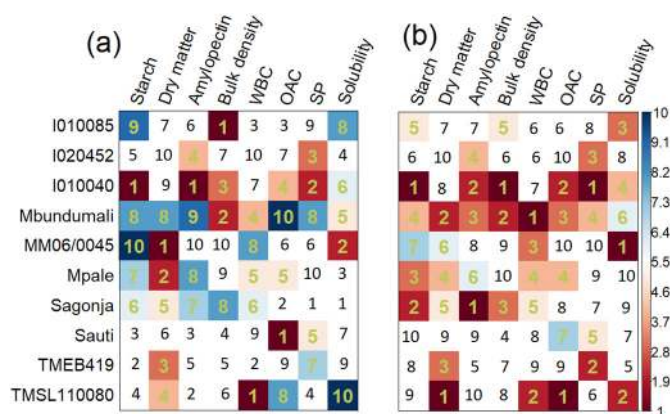


Fig. 7. Biplot for AMMI IPC1 scores of the interaction term ( $G \times E$ ) against means of functional properties (a) Water binding capacity, (b) Swelling power, (c) Oil absorption capacity and (d) solubility of 10 advanced genotypes and varieties, and four environments. WBC = Water Binding Capacity; OAC = Oil Absorption Capacity; IPC1 = Interaction principle component axis 1;  $G \times E$  = Genotype by environment; AMMI = Additive main effect and multiplicative interaction; Genotypes codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.



**Fig. 8.** Summary of results of stability analysis for genotypes (a) with increased stability according to AMMI, (b) with better yield performance and increased stability according to AMMI. Only genotypes that were selected according to Shukla's stability variance and Kang's yield-stability statistics have been shaded with different colours showing the sensitivity of the genotypes, ranging from dark red being the most sensitive to dark blue being the least. The number denotes a rank according to AMMI (a) stability value (ASV) and (b) Yield stability index (YSI) to identify crops with better response and improved stability. WBC = Water Binding Capacity; OAC = Oil Absorption Capacity; SP = Swelling power; AMMI = Additive main effect and multiplicative interaction.

content, amylopectin content, swelling power, and water binding capacity. The appreciable influence of location supports that cassava genotypes' fresh root dry matter content depends on the edaphic-climatic and agronomic conditions. Growing cassava genotypes in well-suited environments will definitely achieve high fresh root dry matter content, starch and amylopectin content, and their associated functional properties. For instance, Mkondezi and Chitala are well suited for genotypes with high fresh root dry matter content, starch and amylopectin content, bulk density and the functional properties, probably because of the role of high temperatures, rainfall and soil organic matter and potassium content in accelerating cassava growth and bulking of tuberous roots.

Based on the principal component analysis, MM06/0045, Mpale, TMEB419 and TMSL110080 were the most stable genotypes for dry matter content, and Chitedze was the most stable site. IO10085, IO10040 and Mbundumali were the most stable genotypes for bulk density, and Mkondezi was the most stable site. For amylopectin and starch, the most stable genotypes were TMSL110080, IO10040, Sauti, TMEB419 and IO20452. Mkondezi and Chitala were the most stable sites, with Chitala presenting a better opportunity for higher starch and amylopectin content. MM06/0045 and IO10085 were the most unstable genotypes for amylopectin and starch and were best suited at Chitedze. Mbundumali and IO10040 were the most selected for both improved stability and better yield performance according to AMMI. IO10040 showed higher starch-related properties (starch and amylopectin content, bulk density, OAC, solubility and swelling power), whereas Mbundumali yielded higher dry matter content and WBC. They are followed by Mpale and Sagonja varieties and MM06/0045 and TMSL110080 genotypes, with TMSL110080 as the highest yielding in dry matter content. To this end, the high-yielding improved genotypes in the trial did not completely outperform the released and local varieties in terms of stability. Generally, most advanced genotypes showed comparable stability but yielded the same functional properties as the released and local varieties. The lack of association between high root yield and stable performance of advanced genotypes suggests further research into the nature of stability of performance of cassava genotypes destined for industrial applications.

#### Declaration of competing interest

The authors declare that they have no conflict of interest.

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