

Genetic parameters, stability and selection of cassava genotypes between rainy and water stress conditions using AMMI, WAAS, BLUP and MTSI

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ABSTRACT

An experiment was conducted with 25 cassava genotypes in Randomized Block Design with three replications in rainy seasons 2017–18 and 2018–19; water stress 2018–19 and 2019–20 to select the stable and superior genotypes that effectively tolerate water stress. AMMI ANOVA indicated significant $G \times E$ interaction for all the traits, Leaf Area Index (LAI), Yield per Plant (YPP), Harvest Index (HI), Dry Matter (DM) and Starch Yield per Plant (SYP). A high environment effect of 68.38 % of the total sum of squares was observed for DM followed by SYP, LAI, YPP and HI. A significant residual variation in $G \times E$ for YPP, HI and DM demonstrated that the presence of considerable noise in the model for these traits. Higher PCV and GCV values, moderate level of broad-sense heritability and high Genetic Advance as per cent of mean was observed for LAI, YPP and SYP indicating the possibility of high genetic gains for these traits through selection. CI-158 for LAI, CR43-11 for YPP, M4 for HI, Sree Pavitra and 9S127 for DM were identified as stable based on mean vs. WAAS biplot. Among all the environments, 8S501 and CR43-7 were ‘all-time winners’ for YPP. The rainy season and water stress were the two mega environments whose influence on traits was different. The accuracy of selection was very high for the traits HI followed by YPP and SYP. 8S501 followed by CR43-7 for YPP; 8S501 followed by CR43-7 for HI; 8S501 followed by Sree Athulya for DM; CI-130 followed by 8S501 for SYP had the highest predicted mean values. Based on MTSI, 8S501 followed by Sree Athulya, CR 43-7 and 9S127 were selected considering their mean performance and stability.

1. Introduction

Starchy tubers of Cassava (*Manihot esculenta* Crantz) are the staple food of the masses across the tropical regions of the world. It is a new world crop believed to be originated in Brazil and spread between N30° and S30° latitudes through human migrations (Byju and Suja, 2020). The global area under cultivation, production and productivity of cassava reached to 24.8 mha, 282.7 mt and 11.5 t/ha respectively in 2018 whereas the area under cultivation and production of cassava in India in 2018 was 0.23 mha and 4.65 mt respectively (FAOSTAT, 2020).

Cassava was introduced to India in the 17th century by Portuguese. It is grown not only for human and animal consumption but also for its starch-based industrial applications. Cassava can be grown throughout the year in a tropical humid climate, but, the limited water availability in the post-monsoons adversely affects leaf area, tuber yield, harvest index, dry matter and starch content of tubers. Cassava is considered as relatively tolerant to drought compared to other crops, but at the expense of tuber yield (Koundinya et al., 2018b). Ravi and Saravanan

(2001a,b), Helal et al. (2013); Carvalho et al. (2016) and Oliveira et al. (2017) also found that water deficit stress caused reduction in the growth, physiological and yield parameters of cassava. Water stress is the most commonly occurring phenomenon, nowadays, worldwide, in the context of climate change. Hence, there is a necessity to breed, evaluate and select superior genotypes continuously against drought stress.

Genetic improvement of crop varieties against abiotic stresses is an important adaptation strategy (Koundinya et al., 2018a). Hybridization breeding is often unsuccessful in cassava due to lack of flowering in superior clones. Some clones produce flowers but protogyny, non-prolific and asynchronous flowering are major the problems (Hegde et al., 2019). Hence, clonal selection is the most commonly used breeding method in cassava. The selection of superior genotypes operates on the exploitation of available or induced genetic variation. Despite its vegetative propagation, cassava maintains huge variability among the germplasm. In fact, vegetative propagation enabled cassava to retain maximum variants without much genetic erosion. Besides

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superior performance, stability is also an important criterion to ensure a steady performance of a genotype over the different environments.

Phenotypic stability analyses are evolving continuously to add precision to the genotype and environmental interaction component. Instead of depending upon a single analysis, papers published on phenotypic stability of various crops often found in a combination of two to three analyses. It is very critical to select the correct method of analysis to capture the maximum genotype (G) and environment (E) interaction. Additive Multiplicative Mean Interaction (AMMI) model is the most commonly used model so far. AMMI analysis is based on the single value decomposition of the G and E interaction which is further used for calculation of the principal components. AMMI is frequently used in combination with GGE (Genotype + Genotype × Environment) to visualize which-won-where and to identify mega environments. Scientists also often argue the merits of these models and vouch for their favourite models. Recently Olivoto et al. (2019a) developed a new concept WAAS (Weighted Average of Absolute Scores) which takes all the PCs in the AMMI ANOVA into account and used it in AMMI I biplot instead of first PC thereby capturing the entire G × E interaction variance. AMMI biplot takes the only first PC leaving a significant amount of interaction variance in remaining PCs.

The focus is now shifting towards Linear Mixed-effects Models (LMM) where the effects of block and environment are fixed and the genotype effect is considered as random, thus G × E. LMM offers a prediction component, Best Linear Unbiased Predictor (BLUP), which predicts the performance of a genotype in a given environment. Weighting between Weighted Average Absolute Scores from BLUP (WASSB) and mean value (Y) facilitates the simultaneous selection for yield and stability through a superior index called WASSBY (Olivoto et al., 2019a).

Stability of genotypes is measured, in general, based on a single trait. Selection of stable superior genotype for more number of traits is needed in combination breeding. MTSI (Multi Trait Stability Index) identifies the stable genotypes by considering all the traits into consideration (Olivoto et al., 2019b). This index is much useful to the breeders for the selection of genotypes that are superior for multiple traits. This index would be an appropriate statistic for ideotype breeding because MTSI is the difference of the distance between a genotype and an ideotype. In this paper, these analyses are used to find out the phenotypic stability of cassava genotypes and selection of stable superior genotypes based on multiple traits under rainy and water stress conditions in the tropical humid climate of Kerala, India.

2. Materials and methods

2.1. Experimental unit, location and material

The experimental site is located in the Block-IV of ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. The experimental unit is red laterite soils with acidic pH (4.32). The landscape was hilly terrain where the topsoil was eroded and poor in nutrient status. The available organic Carbon, Nitrogen, Phosphorous and Potassium were 0.42 %, 210.11 kg/hectare, 21.49 kg/hectare and 180.99 kg/hectare respectively. The experimental material comprised of 25 cassava genotypes from India, Malaysia and South America and some of them are hybrid clones.

2.2. Experimental design

The experiment was carried out in four conditions viz., rainy season 2017–18, rainy season 2018–19, water stress 2018–19 and water stress 2019–20 in a Randomized Block Design with three replications at spacing of 90 cm between rows and between plants. In each genotype, in each treatment (rainy and water stress) and each replication 10 stakes were planted.

For the rainy season crop, the stakes were planted in May with the

onset of monsoons and harvesting was done in December-January. This crop was under irrigation continuously for seven months as it received a significant amount of SW and NE monsoon rainfall which was sufficient to attain full growth of cassava. For the water stress crop, the stakes were prepared and planted in the soil in the last week of August. This crop was under irrigation up to 3 months after planting. By the end of November, after completion of the NE monsoon rainfall, irrigation was withdrawn, the dry period began and continued up to the harvesting of the crop in April. All the recommended cultural practices were followed as per Ravindran et al. (2013) in both the treatments except the irrigation.

2.3. Weather condition

The weather in the experimental site is tropical humid climate favourable for the cultivation of cassava. The weather parameters recorded during the experimental period are given in Table 1. There were not many differences among environments for maximum temperature, minimum temperature and maximum relative humidity. It was clearly understood from the table that amount of total rainfall and number of rainy days were very less in mid-period of environment 3 and 4 causing water stress on the crop growth. The number of rainy days was very low i.e. only 5 and 10 in mid-period of environments 3 and 4 amounting for a very less rainfall of 66.4 and 188.0 mm respectively when compared to the environments 1 and 2 which had 60 and 51 rainy days and 979.3 and 980.6 mm rainfall respectively. The minimum relative humidity was also low in the mid-period of environments 3 and 4 (51.7 and 56.1 %) in comparison with environments 1 and 2 (80.4 and 67.6 %).

2.4. Observations

Observations were recorded on Leaf Area Index (LAI), Yield per Plant (YPP), Harvest Index (HI), Dry Matter (DM) and Starch Yield per Plant (SYP). LAI was measured as the ratio of total leaf area to land area at 6 months after planting. Leaf area was measured using LI-3000C (Li-Cor Bioscience) Leaf area meter.

$$\text{LAI} = \frac{\text{Total Leaf Area}}{\text{Land Area (Spacing)}}$$

YPP (kg/plant) was measured by weighing each plant fresh tuber weight at the time of harvest at 8 months after planting. HI was calculated as the ratio of fresh tuber yield to total biological yield (fresh tuber yield + above ground shoot and leaf weight).

$$\text{HI} = \frac{\text{Tuber yield per plant}}{\text{Total biological yield per plant}}$$

Fresh tubers were chopped into small pieces and weight was recorded. The samples were dried in an oven for 72 h at 60°C. Then the weight of the dried sample was noted. DM was calculated as per cent of fresh tuber weight.

$$\text{DM (\%)} = \frac{\text{tuber dry weight} \times 100}{\text{tuber fresh weight}}$$

Starch content in tubers was estimated as per Sadasivam and Mankam (1991) by using anthrone reagent on a dry weight basis. SYP was calculated as follows:

$$\text{SYP (g / plant)} = \frac{\text{YPP} \times \text{starch per cent}}{100}$$

2.5. Statistical analysis

2.5.1. Additive multiplicative mean interaction model (AMMI)

The data obtained from various parameters were subjected to the AMMI analysis as per the statistical model given by Zobel et al. (1988). The mean sums of squares from the AMMI ANOVA were used to

Table 1

Weather parameters during experimentation.

Environment	Stage of the crop	Max T (°C)	Min T (°C)	Mean T (°C)	Max RH (%)	Min RH (%)	RF (mm)	NRD
Environment 1 (Rainy 2017–18)	Early Stage (0–3 Months)	32.2	24.9	28.6	90.9	78.3	560.4	40.0
	Mid Stage (4–8 Months)	31.3	24.3	27.8	93.7	80.4	979.3	60.0
Environment 2 (Rainy 2018–19)	Early Stage (0–3 Months)	30.2	23.5	26.9	99.3	75.3	653.8	46.0
	Mid Stage (4–8 Months)	31.0	23.1	27.1	99.5	67.6	980.6	51.0
Environment 3 (Water Stress 2018–19)	Early Stage (0–3 Months)	31.4	23.2	27.3	99.6	66.3	570.9	33.0
	Mid Stage (4–8 Months)	32.7	22.8	27.8	95.6	51.7	66.4	5.0
Environment 4 (Water Stress 2019–20)	Early Stage (0–3 Months)	31.1	24.5	27.8	94.0	82.1	712.6	46.0
	Mid Stage (4–8 Months)	32.7	22.9	27.8	95.7	56.1	188.0	10.0

Max T = Maximum temperature; Min T = Minimum temperature; RH = Relative Humidity; RF = Rainfall; NRD = Number of rainy days (>1 mm).

calculate various genetic parameters such as Genotypic Co-efficient Variation (GCV) and Phenotypic Co-efficient of Variation (PCV) as per Burton (1952), Broad sense Heritability (h^2_{bs}) as per Hanson et al. (1956), Genetic Advance as per cent Mean (GAM) as given by Johnson et al. (1955). In the below formulas, r = number of replications and E = number of environments.

$$\text{Genotypic Variation } (\sigma_g^2) = \frac{\text{MSS due to genotypes} - \text{MSS due to } G \times E - \text{MSS due to error}}{r \times E}$$

$$\text{GCV} = \frac{\sigma_g}{\text{Mean}}$$

$$\text{Phenotypic Variation } (\sigma_p^2) = \sigma_g^2 + \frac{\text{MSS due to } G \times E}{r \times E} + \frac{\text{MSS due to error}}{r}$$

$$\text{PCV} = \frac{\sigma_p}{\text{Mean}}$$

$$h^2_{bs}(\%) = \frac{\sigma_g^2 \times 100}{\sigma_p^2}$$

$$\text{GAM} = \frac{\sigma_p \times h^2_{bs} \times 2.06 \times 100}{\text{Mean}}$$

2.5.2. Weighted average absolute scores (WAAS)

The sum of squares for $G \times E$ Interaction was subjected to single value decomposition. Weighted Average Absolute Scores (WAAS) were calculated from the IPCAs of $G \times E$ Interaction from AMMI ANOVA. The IPCA1 in the traditional AMMI1 biplot was replaced by WAAS (Olivoto et al., 2019a).

$$\text{WAAS}_i = \frac{\sum_{k=1}^p |\text{IPCA}_{ik} \times \text{EP}_k|}{\sum_{k=1}^p \text{EP}_k}$$

Where WAAS_i = weighted average of absolute scores of the ith genotype or environment; IPCA_{ik} = absolute score of the ith genotype or environment in the kth IPCA; and EP_{ik} = magnitude of the variance explained by the kth IPCA.

2.5.3. Which-won-where graph

A graphic representation of which-won-where pattern and delineation of mega environments was done by plotting the nominal mean values against environmental IPCA1.

$$\hat{Y}_{ij} = \mu_i + \text{IPCA1}_i \times \text{IPCA1}_j$$

Where, \hat{Y}_{ij} = nominal mean for ith genotype in the jth environment, μ_i = grand mean of the ith genotype, IPCA1_i = IPCA1 score of the ith genotype, IPCA1_j = IPCA1 score of the jth environment.

2.5.4. Best linear unbiased predictor (BLUP)

A linear mixed model was employed by considering the genotypic

effects as random. The variance components were estimated by restricted maximum likelihood (Dempster et al., 1977). The significance of random effects (genotype and $G \times E$) was tested by the likelihood ratio test. The Best Linear Unbiased Predictor (BLUP) of ith genotype was predicted as ($\text{BLUP}_i = \mu + \hat{g}_i$) the sum of the general mean overall environments and genotypic effect.

$$\text{Genotypic effect}(\hat{g}_i) = h^2_g(\bar{Y}_i - \bar{Y})$$

Weighted Average Absolute Scores from BLUP (WASSB) were estimated based on single value decomposition of the GEI effects from the matrix of the BLUP. The formula is similar to WAAS. Simultaneous selection index for each genotype (WAASBY) was calculated allowing weighting between its mean performance and stability as given below (Olivoto et al., 2019b).

$$\text{WAASBY}_i = \frac{(rY_i \times \theta_Y) + (rW_i \times \theta_s)}{\theta_Y + \theta_s}$$

Where, θ_Y and θ_s are the weights for the response variable and WAASB (65 and 35 % giving more weight to the mean performance than stability) and rY_i and rW_i are the rescaled values 0–100 for response variable and WAASB respectively.

2.5.5. Multi trait stability index (MTSI)

MTSI is based on the genotype–ideotype distance (Euclidian) using the scores obtained in factor analysis (Olivoto et al., 2019b). MTSI was calculated based on all the yield and quality traits viz., YPP, HI, DM and SYP. LAI was not considered because of its lack of any correlation with yield components. A Varimax rotation criterion was applied for calculating the final loadings. The genotype scores were obtained using standardized WAASBY means. The ideotype scores were also obtained considering that an ideotype has the highest WAASBY values (100) for all the observed traits. MTSI was calculated as follows (Olivoto et al., 2019b):

$$\text{MTSI} = \left[\sum_{j=1}^f (F_{ij} - F_j)^2 \right]^{0.5}$$

Where F_{ij} = jth score of the ith genotype; F_j = jth score of the ideotype.

Table 2
Analysis of Variance through AMMI model.

Source of Variance	df	LAI		YPP		HI		DM		SYP	
		MSS	% SS	MSS	% SS	MSS	% SS	MSS	% SS	MSS	% SS
ENV	3	20.80*	36.60	18.87*	34.80	0.99*	27.74	8394.19*	68.38	1760000.00*	47.80
GEN	24	1.79*	25.10	2.13*	31.40	0.15*	34.30	89.46*	5.83	112000.00*	24.40
GEN:ENV	72	0.43*	18.00	0.35*	15.33	0.02*	13.43	42.61*	8.33	19900.00*	12.95
PC1	26	1.15*		0.47*		0.03*		87.87*		50100.00*	
PC2	24	0.03		0.38*		0.01*		32.40*		5230.00*	
Residuals	22	0.00		0.16*		0.01*		0.24*		33.70	
Error	192	0.02		0.02		0.01		17.39		1020.00	
Total	371	0.46		0.44		0.03		99.26		29800.00	
		Var (%)	Cum Var (%)	Var (%)	Cum Var (%)	Var (%)	Cum Var (%)	Var (%)	Cum Var (%)	Var (%)	Cum Var (%)
PC1		97.7	97.7	49.5	49.5	63.3	63.3	74.5	74.5	91.2	91.2
PC2		2.2	99.9	36.2	85.7	20.4	83.7	25.3	99.8	8.7	99.9
Residual		0.1	100.0	14.3	100.0	16.4	100.0	0.2	100.0	0.1	100.0

MSS = Mean sum of squares; SS = Sum of squares; df = Degrees of freedom; Var = Variance; Cum Var = Cumulative Variance; PC = Principal Component; * = Significant at 5 % level.

2.6. Software

All the statistical analyses were done using R open-source software 4.0.2 using the package 'metan' developed by Olivoto (2019).

3. Results and discussion

3.1. AMMI ANOVA

AMMI ANOVA displayed significant interactions for genotypes, environment and their interaction for all the characters (Table 2). A high environment effect of 68.38 % of the total sum of squares was observed for DM followed by SYP (47.80 %), LAI (36.60 %), YPP (34.80 %) and HI (27.74 %). Very low genotype effect was observed for DM (5.83 % of the total sum of squares) while high genotype effect was observed for HI (34.30 %). The interaction effect ($G \times E$) ranged from low (8.33 %) for DM to medium (18.00 %) for LAI. The $G \times E$ was further partitioned into Interaction Principal Component Axes (IPCA) and residuals. The IPCA1 was significant and contributed highly 97.7 % variation for LAI. The IPCA1 and IPCA2 were significant and cumulatively explained 99.9 % $G \times E$ variation for SYP, 99.8 % for DM, 85.7 % YPP and 83.7 %.

Various genetic parameters were estimated based on AMMI ANOVA of multi-seasonal data (Table 3). Higher PCV and GCV values were observed for SYP, LAI and YPP. Moderate level of broad-sense heritability (h^2_{bs}) was observed for LAI (43.80 %), YPP (55.49 %), HI (59.79 %) and SYP (53.05 %) whereas low heritability was recorded for DM (13.56 %). High Genetic Advance as per cent of mean (GAM) was observed for SYP (73.10 %) followed by YPP (61.20 %) and LAI (51.85 %) whilst moderate GAM was recorded on HI (38.13 %) and very low GAM was found for DM (4.14 %).

3.2. Mean vs. WAAS biplot

Mean vs. WAAS biplot, simply called as WAAS biplot, was

constructed as x-axis being the mean values and y-axis being the WAAS scores of the genotypes (Fig. 1). The genotypes identified from WAAS biplot into different stability groups are presented in Table 4. A detailed explanation is provided in the discussion section on how the genotypes were identified as stable, fairly stable and suitable for favourable conditions from WAAS biplot in section 4.2.

From the WAAS biplot of LAI, the most stable genotype for LAI was *Sree Visakham* and CI-158 with WAAS value zero, but, CI-158 alone with mean value more than average (>0.88) was qualified for general cultivation. Similarly, the stable genotype identified based on WAAS biplot for YPP was CR43-11. It had WAAS value near to zero and the mean tuber yield was just below the average (0.95 kg/plant). The fairly stable genotypes with above average yields were 8S501, CR 43-7, H-226, 9S127 and CI-130. Two genotypes were found as stable genotypes for HI based on WAAS score viz., *Sree Prakash* and M4, but only M4 had the average mean value (0.43). The stable genotypes for DM were *Sree Pavitra* and 9S127. But only 9S127 had mean values above the average (>28.69 %). None of the genotypes was qualified as stable as no genotype had recorded WAAS value zero or near to zero for SYP. All the genotypes were unstable for SYP and were highly influenced by the environment. The genotypes 9S127, 8S501, *Sree Athulya*, CR 43-7 and CR 43-2 were identified as fairly stable for the majority of the characters.

3.3. Which-won-where and mega environments

Which-won-where pattern of the genotypes based on predicted yields in each environment and delineation of mega environments were represented by Fig. 2. The genotype CI-174 performed well in environments 1 and 2 while the genotype CI-96 won in the environments 3 and 4 for LAI. Among all the environments, 8S501 and CR43-7 won for YPP making them as 'all-time winners' for YPP. For HI, the genotypes with high predicted mean values were different for all the four environments. CR43-7 had the highest predicted mean value in environment 1, CR43-7 and 8S501 in environment 2, CR43-7 in environment 3 and CI-421A in environment 4. There was no 'all-time winner' found for DM also. CR43-11 and *Sree Athulya* had high predicted DM in environments 1 and 2 respectively. In environments 3 and 4, 8S501 had the highest predicted mean values. The genotype CI-130 was found as 'all-time winner' for SYP.

For LAI, YPP and SYP the two rainy seasons and the two water-stress environments were fell apart at different ends of the graph on x-axis. The distance between environments 1 and 2 was slightly higher for DM, but environments 3 and 4 were close to each other. But, for HI, the four seasons were at distant from each other.

Table 3
Genetic parameters estimated from AMMI ANOVA.

Parameter	LAI	YPP (kg/plant)	HI	DM (%)	SYP (g/plant)
Genotypic Co-efficient of Variation (%)	38.03	39.88	23.94	5.46	48.73
Phenotypic Co-efficient of Variation (%)	57.46	53.53	30.96	14.83	66.90
Heritability (broad sense) (%)	43.80	55.49	59.79	13.56	53.05
Genetic Advance	0.46	0.59	0.16	1.19	130.71
Genetic Advance as per cent Mean (%)	51.85	61.20	38.13	4.14	73.10

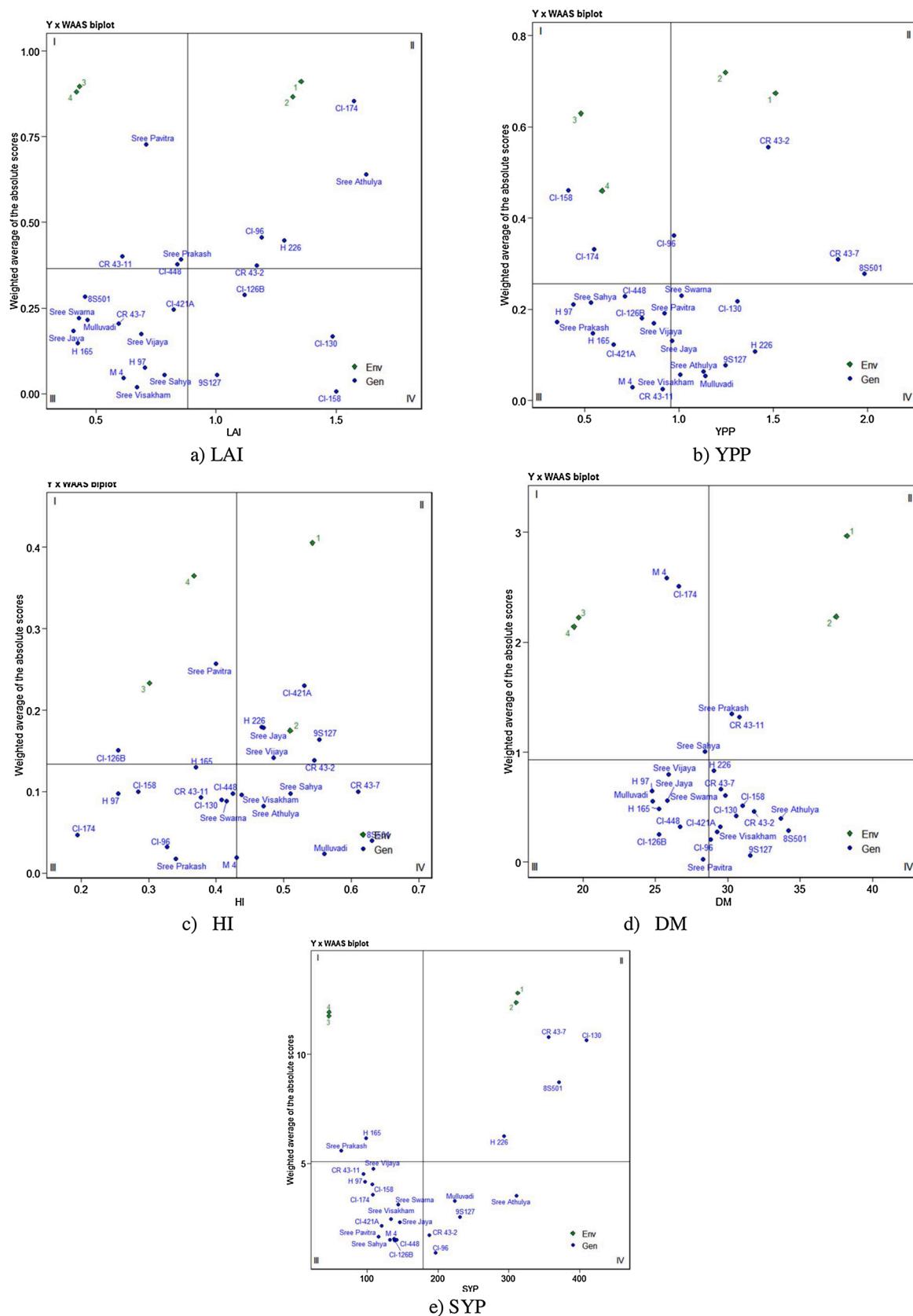


Fig. 1. Mean vs. WAAS biplot from AMMI ANOVA.

Table 4
Genotypes identified for various conditions based on Mean vs. WAAS biplot.

S. No	Trait	Category	Genotypes
1	LAI	Stable and Better performance	CI-158
		Fairly stable and Better performance	9S127, CI-130
		Better performance in the rainy season	CI-174, Sree Athulya
2	YPP	Stable and Better performance	—
		Fairly stable and Better performance	8S501, CR 43-7, H-226, 9S127, CI-130
		Better performance in the rainy season	CR 43-2
3	HI	Stable and average performance	M4
		Fairly stable and Better performance	8S501, CR43-7, Mulluvadi, 9S127, CR43-2, Sree Athulya
		Better performance in the rainy season	—
4	DM	Stable and average performance	9S127
		Fairly stable and Better performance	8S501, Sree Athulya, CR 43-2
		Better performance in the rainy season	—
5	SYP	Stable and average performance	—
		Fairly stable and Better performance	Sree Athulya, H-226, Mulluvadi, 9S127
		Better performance in the rainy season	CI-130, 8S501, CR 43-7

3.4. LMM-Best linear unbiased predictor

A perusal of Table 5 indicated that genotype (G) and G × E variances were significant for all the traits based on likelihood ratio test. The contribution of environment variation was high for DM (58.19 %) followed by HI (27.52 %). The coefficient of determination (R^2_{GEI}) of G × E interaction was found to be moderate to low.

The accuracy of selection was very high for the traits HI (0.933) followed by YPP (0.915) and SYP (0.907). High selection accuracy was found for LAI (0.873) and moderate accuracy was observed for DM (0.724). The genotypic correlation among environments (r_{ge}) was high for LAI (0.871), SYP (0.861) and YPP (0.811) while it was low for HI (0.445) and DM (0.326).

The predicted mean values of the genotypes are presented in Fig. 3. The predicted mean values and the genotypes were plotted on a biplot. Ten genotypes had above-average predicted mean value for LAI. *Sree Athulya* followed by CI-174, CI-158 and CI-130 had the highest predicted mean values for LAI. Out of 12 genotypes that had above-average predicted mean value, 8S501 followed by CR43-7 had the highest predicted means for YPP. Similarly, 13 genotypes for HI, 14 genotypes for DM and 9 genotypes for SYP had above-average predicted mean values. 8S501 followed by CR43-7; 8S501 followed by *Sree Athulya*; and CI-130 followed by 8S501 had the highest predicted mean values for HI, DM and SYP respectively.

3.5. Multi trait stability

MTSI was calculated based on all the yield and quality traits viz., YPP, HI, DM and SYP. LAI was not considered because of its lack of any correlation with yield components. A Varimax rotation criterion was applied for calculating the final loadings. The first two factors having eigenvalue more than 1.00 i.e. PC1 representing 63.2 % variation was selected (Table 6). After Varimax rotation, the communality was ranged from 0.423 for DM to 0.755 for YPP with a mean value of 0.632.

Fig. 4 shows the MTSI values of the genotypes. The genotypes

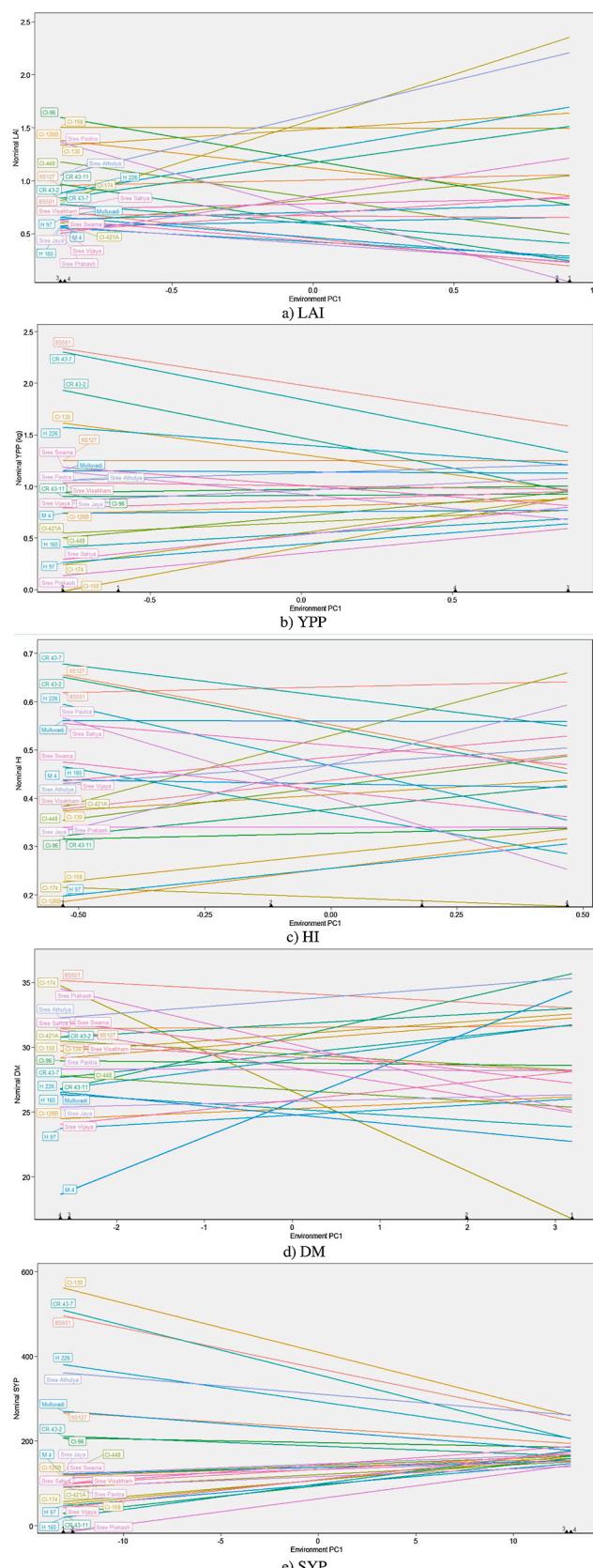


Fig. 2. Which-won-where plots based on predicted yields in each environment.

Table 5
Estimation of variance components from LMM.

	LAI	YPP	HI	DM	SYP
GEN	0.113**	0.149**	0.0111**	3.9**	7704**
GEN:ENV	0.136**	0.107**	0.00469**	8.6**	6280**
Error	0.020	0.025	0.006	17.4	1016
% GV in PV	42.01	53.02	50.92	13.04	51.36
%IV in PV	50.56	38.08	21.56	28.76	41.87
%EV in PV	7.43	8.90	27.52	58.19	6.77
PV	0.269	0.281	0.0218	29.9	15,000
R ² _{GEI}	0.504	0.382	0.217	0.283	0.419
A _s	0.873	0.915	0.933	0.724	0.907
r _{ge}	0.871	0.811	0.445	0.326	0.861

GV = Genotypic variation; IV = Interaction variance; PV = Phenotypic variation; EV = Environment variation; R^2_{GEI} = co-efficient of determination of GE interaction; As = Accuracy of selection; r_{ge} =genotypic correlation across environments; **=significant at 1% level based on likelihood ratio test.

indicated in red colour dots were selected based on their MTSI values at a selection intensity of 15 %. The stable and selected genotypes in the order were 8S501 followed by *Sree Athulya*, CR 43-7 and 9S127. A selection differential of 62, 31.5, 12.3 and 77.5 % for YPP, HI, DM and SYP was observed respectively by the selection of these four genotypes.

4. Discussion

4.1. Variance components

It can be inferred from the AMMI ANOVA that significant differences were observed for genotypes, environment and their interaction for all the characters confirming the influence of environment on the phenotypic expression of traits (Table 2). The high environment effect for all the traits except HI signified that these traits were under the influence of the environment. It was higher than the genotype effect and $G \times E$ effect for all the traits except for HI. The low environment effect than genotype for HI suggested that genotype effect played a major role than the environment. The trait, DM was highly influenced as the difference between genotype and environment effect was very high. A significant $G \times E$ interaction in cassava was previously observed by Kvitschal et al. (2006); Morais et al. (2017) and Adjepong-Danquah et al. (2017).

The high percentage of variation explained by IPCA1 and IPCA2 for SYP, DM, YPP and for HI implied that a high proportion of $G \times E$ variance was covered by the first two PCs. Nevertheless, a significant residual variation in $G \times E$ for YPP, HI and DM demonstrated that the presence of considerable noise in the model for these traits. Kvitschal et al. (2006); Morais et al. (2017) observed that non-significant residual

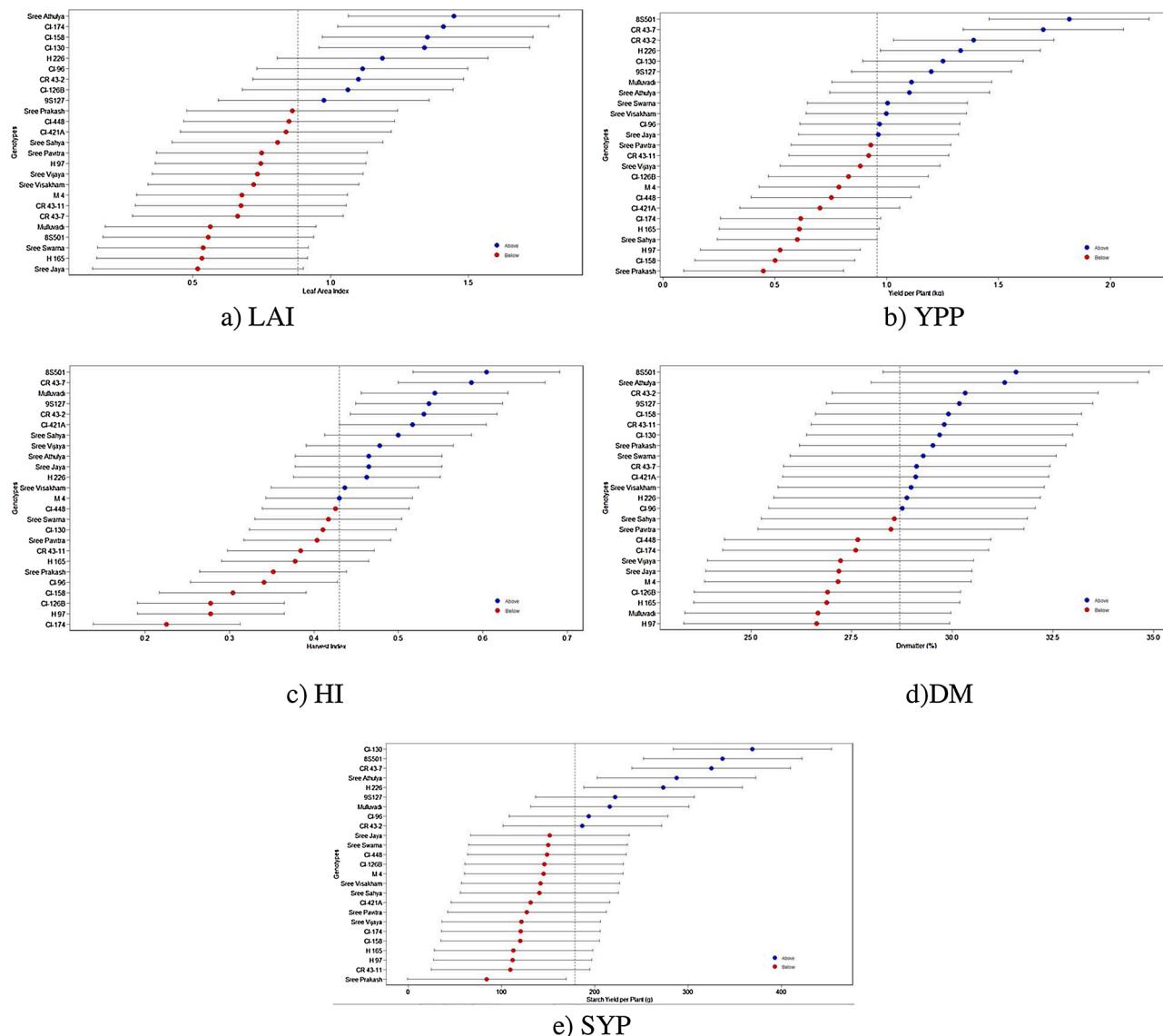


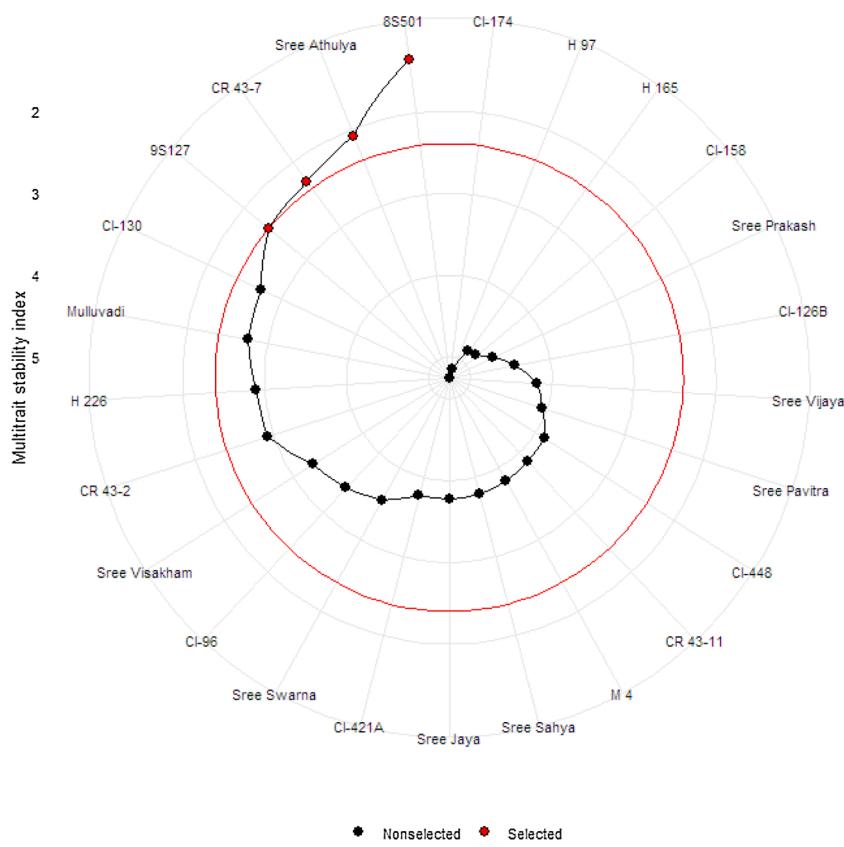
Fig. 3. BLUP mean values of genotypes.

Table 6

Communalities and selection differential of various characters based on MTSI.

S.No	VAR	FA1	Communality	Uniqueness	Xo	Xs	SD	SD (%)
1	YPP (kg/plant)	-0.869	0.755	0.245	0.957	1.55	0.594	62
2	HI	-0.788	0.62	0.38	0.43	0.566	0.136	31.5
3	DM (%)	-0.651	0.423	0.577	28.7	32.2	3.53	12.3
4	SYP (g/plant)	-0.854	0.73	0.27	179	317	139	77.5

Xo = mean of genotypes; Xs = mean of selected genotypes; SD = selection differential.

**Fig. 4.** MTSI values of the genotypes.

interaction for storage root yield of cassava.

Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient variation (GCV) which signified the effect of environment on the expression of traits. A higher difference of 19.43 between PCV and GCV for LAI made it as the most influenced trait by environment followed by SYP (18.17) and YPP (13.65). The traits that were less influenced were HI (7.02) and DM (9.37). High heritability coupled with high genetic advance as per cent of the mean of SYP, YPP and LAI indicated the possibility of high genetic gain by selecting the top 5% genotypes. A very low GAM of DM pointed out that the low improvement could be made by selection for this trait. The present results corroborated with [Adjepong-Danquah et al. \(2017\)](#) for the majority of traits except for high h^2_{bs} for DM. High phenotypic and genotypic coefficient of variation, broad-sense heritability coupled with GAM for SYP, LAI and YPP implied that improvement can be made for these traits through selection. [Darschan et al. \(2017\)](#) also identified traits responsive for selection based on these parameters in cassava.

4.2. Stability of genotypes

WAAS biplot ([Fig. 1](#)) explains the stability based on all IPCA scores unlike to AMMI which considers the only IPCA1. So, WAAS considers the entire $G \times E$ interaction variance in identifying the stable genotypes.

Genotypes with WAAS values zero or near to zero are considered as the most stable ([Olivoto et al., 2019a](#)). But the ideal genotypes for general cultivation would be those having WAAS value zero or near to zero and mean value above the grad mean.

All stable genotypes are not necessarily high yielders. If any genotype performs well in a particular environment, it would always be better to recommend that genotype for that particular environment to exploit its maximum genotypic potential. The genotypes present in different quadrants of biplot could be classified as per their suitability to different environments. The genotypes present in the first quadrant of the biplot have high WAAS values and lower mean values indicating their highly responsive nature to the environment and below-average performance. These genotypes are generally not recommended for general cultivation. The genotypes present in the second quadrant of the biplot are possessing high WAAS and high mean values of a given trait. These genotypes are also highly responsive to the environment but have the good discriminative ability. Under favourable conditions i.e. rainy season crop in this experiment, their performance would be very high. These genotypes can be recommended for cultivation in the periods with good rainfall and high soil moisture availability.

The genotypes located in the third quadrant of biplot had less WAAS indicating their stability or non-responsiveness to the environment yet low productivity. The genotypes present in the fourth quadrant of biplot

had lower WAAS and above-average mean values. The genotypes in this quadrant could be fairly stable and perform well in both the seasons due to their low discriminative ability and optimum productivity. The genotypes having moderate WAAS score (located on both sides of the horizontal line in the graph) with very high mean values, sometimes, would also be expected to perform fairly well under rainy and water stress conditions. Their high trait means would compensate for the reduction under water stress condition. The genotypes suitable for various conditions based on the above explanation can be seen in Table 4. Among all the genotypes, 9S127 and 8S501 were identified as stable or fairly stable for the important characters such as LAI, YPP, HI and DM. Hence, these genotypes could be recommended for general cultivation. Previously, Koundinya et al. (2019) also classified genotypes into different suitable categories based on AMMI1 biplot. AMMI1 biplot is suitable for this type of classification because of the presence of positive and negative IPCA1 values on the y-axis. The sign indicates the positive or negative interaction of the genotype with the environment. Due to the absolute scores on the y-axis in WAAS biplot, it is difficult to say the direction of the interaction. The genotypes with negative IPCA1 values are negatively correlated with the environment. It gives the understanding that those genotypes with negative IPCA1 value and high trait mean have positive response to the negative environments (Koundinya et al., 2019). Those genotypes can be recommended for environments with poor growing conditions/resources. Hence, the use of absolute values does not yield this information.

4.3. $G \times E$ interaction from WAAS biplot

Genotype and environment interaction can also be inferred from mean vs. WAAS biplot. If the environments are flocked together on the biplot, their interaction with the genotypes is similar. Higher the WAAS value of an environment indicates its higher interaction with the genotypes. If the environmental means are above average, their influence on the traits is positive. The below-average environment-mean values signify the negative influence of the environment.

For LAI and SYP, the environment 1 and 2 were flocked together in the second quadrant of the biplot. Similarly, environment 3 and 4 were located together in the first quadrant of biplot. The very high WAAS values of all environments indicated that their influence on the genotypes was very high. Environment 1 and 2 i.e. the rainy seasons of 2017–18 and 2018–19 interacted similarly with the genotypes for LAI and SYP. Likewise, the interaction of environment 3 and 4 (water stress of 2018–19 and 2019–20) with the genotypes was also similar. The influence of environment 1 and 2 were positive on LAI and SYP while it was negative for environment 3 and 4.

For YPP, the interaction of environments 3 and 4 was different. Environment 1 and 2 also interacted slightly differently and positively. The influence of environment 2 (rainy seasons of 2018–19) was high on the genotypes than environment 1 (rainy seasons of 2017–18) and environment 3's (water stress of 2018–19) influence was higher than environment 4 (water stress of 2019–20) based on their WAAS numbers for YPP. Similarly, the influence of rainy seasons of 2017–18 was higher on the genotypes than rainy seasons of 2018–19 for DM. But, the interaction of water stress of 2018–19 and 2019–20 was quite similar.

For all the traits studied, the influence of environments 1 and 2 (rainy seasons of 2017–18 and 2018–19) was positive with above-average mean values suggesting that the rainy season favoured the leaf area index, crop growth, tuber yield per plant, dry matter content and starch yield per plant while water stress of 2018–19 and 2019–20 caused a negative impact on these traits.

Unlike to other traits, in case of HI, the positive interaction of environments 1 and 2 was different. The WAAS of environment 1 was higher than environment 2. The influence of rainy season 2017–18 on phenotypic expression of HI was higher than rainy season 2018–19. Similarly, the interaction of genotype and environment was different for environments 3 and 4. The WAAS of environment 4 (water stress

2019–20) was higher than environment 3 (water stress 2018–19) which meant that the influence of environment 4 was higher than environment 3.

Harvest Index (HI) was calculated as the ratio of tuber yield to tuber yield + above ground yield. The difference between environments 1 and 2 (rainy seasons of 2017–18 and 2018–19) of HI was higher than YPP. It suggested that the influence of environments 1 and 2 was also different for above-ground growth. Same was the case with environments 3 and 4 (water stress 2018–19 and 2019–20).

4.4. Which-won-where and mega environments

Generally, GGE (Genotype + Genotype \times Environment) biplot, commonly known as 'which-won-where', is used to identify the genotypes which performed well in each environment. They are also used to identify mega environments. To represent the same, Olivoto et al. (2019a) provided a graphic interpretation by plotting the trait nominal means (\hat{y}_{ij}) against environmental IPCA1 values (Fig. 2). The comparison of GGE biplot (not given in this paper) and environment vs. nominal mean biplot showed similar results. The winner is the genotype which has high nominal trait mean value in a given environment. The lines in the graph indicate how a genotype's predicted mean performance is changing over the environments.

The rainy season favoured the genotype CI-174 to maintain high LAI, but CI-96 maintained high LAI under water stress conditions due to its less reduction in leaf area. The prediction of 8S501 and CR43-7 as 'all-time winners' for YPP was quite justified by their high tuber yield per plant among all the seasons in the present experiment. In rainy season 2017–18 and 2018–19, CR43-2 recorded third highest predicted yield, but it was reduced in water stress 2018–19 and 2019–20 depicting its susceptibility to water stress.

High nominal mean values of CR43-7 and 8S501 for HI was due to their higher tuber yields. In the fourth environment i.e. water stress 2019–20, though CI-421A produced less tuber yield (0.381 kg/plant), its above-ground growth was very low (1.02 kg/plant) resulting in high HI values. This could be the reason for its prediction in environment 4 as highly predicted genotype for HI than other high yielding genotypes such as 8S501 and CR43-7 whose tuber yield in water stress 2019–20 was 1.681 and 1.259 kg/plant respectively.

There was no 'all-time winner' for DM showing the genotypes interacted differently in all four environments. Interestingly, CI-174 had differential predicted means. It was the lowest and second-lowest genotype for DM in environments 1 and 2, but, it was the second-highest yielding genotype in environments 3 and 4. This indicated that CI-174 was performed solely in water stress environments for DM. The genotype *Sree Pavitra* had horizontal line depicting its stability among all the environments. WAAS biplot also showed the stable nature of *Sree Pavitra* for DM.

The predicted SYP was less for the majority of the genotypes viz., M4, *Sree Jaya*, CI-448, CI-126B, *Sree Swarna*, *Sree Sahya*, *Sree Visakham*, CI-174, CI-421A, *Sree Pavitra*, H-97, *Sree Vijaya*, CI-158, H-165, CR43-11 and *Sree Prakash* in rainy season 2017–18 and 2018–19 than water stress 2018–19 and 2019–20. This suggested that the rainy season had a negative impact on the starch content of the majority of the genotypes.

These plots can also be used for the identification of mega environments. The distance between environments is used to delineate the mega environments. The environments 1–4 were marked on the x-axis. If the environments are pointed closely, they are similar. For LAI, YPP and SYP the two rainy seasons interacted similarly with the genotypes likewise two water stress environments. There was a clear difference in the way of interaction of these two types of environments on the expression of these traits. Though environments 1 and 2 were rainy seasons, there was a slight difference between them for DM. In the case of HI, all the four seasons were had different interaction with genotypes. As mentioned earlier, HI is the ratio of tuber yield to biological yield, it could be said that each of the four environments differently influenced the above-

ground growth of cassava. It could be said that the rainy season and water stress were two different mega environments for LAI, YPP and SYP, but, there were variations even in similar environments over the years for DM and HI. The environment interactions were not same for all the traits.

4.5. LMM-Best linear unbiased predictor

AMMI ANOVA is based on a fixed-effects model where genotype and environment effects are considered as fixed. Mixed models usage in the estimation of $G \times E$ interaction is advocated by Piepho (1994) considering the genotypic effects as random. A linear mixed-effects model was employed wherein genotype effect was considered as a random effect, thereby $G \times E$. Taking the genotype and environment effects in a mixed model approach, BLUP (Best Linear Unbiased Predictor) is more advantageous to plant breeders because it results in more accurate predictions of genotypes future mean values. It has a more predictive accuracy of random effects and better deals with unbalanced or incomplete data (Smith et al., 2005).

Analysis of variance through a Linear Mixed Model also indicated the significant differences among genotypes and their interaction with an environment similar to AMMI ANOVA. The per cent of genotypic variation was higher than environment variation in phenotypic variation for the majority of the traits except for DM. Albeit the contribution of the environment is less for other traits, it was having a significant effect on the phenotypic expression of traits. Low R^2_{GEI} of HI, DM, YPP and moderate R^2_{GEI} of SYP and LAI demonstrated the presence of high residual variation in $G \times E$ interaction component contrasting to AMMI ANOVA which explained high proportion of $G \times E$ interaction through first two PCAs.

The genotypic accuracy of selection (A_s), in other terms predictive accuracy of the model, is the correlation between observed and predicted values (Olivoto et al., 2019a). Moderate to very high A_s values for all the traits indicated the reliability of the model in the selection of superior genotypes. The high genotypic correlation among environments (r_{ge}) LAI, SYP and YPP suggested the similar trend across all the environments and easy identification of stable and superior genotypes while the low r_{ge} for HI and DM indicated difficulties in the selection of superior stable genotypes for these traits and need for detailed accurate information for selecting stable superior genotypes. Similar observations were made by Sousa et al. (2019) in cowpea for immature seed yield. The genotype 8S501 had high BLUP mean values for YPP, HI, DM and SYP made it as one of the sought after genotypes for better performance.

4.6. Selection of genotypes based on stability and mean performance

Multi Trait Stability Index (MTSI) based on WAASBY put forward by the Olivoto et al. (2019b) indicates the stable genotypes based on multiple traits. MTSI would help select the genotypes based on the stability of multiple traits and mean performance. MTSI is based on the genotype-ideotype distance (Euclidian) using the scores obtained in factor analysis. The ideotype has the highest WAASBY values for all the observed traits (Olivoto et al., 2019b).

The lower values of MTSI indicate stable genotypes based on multiple traits. The stable and selected genotypes in the order were 8S501 followed by *Sree Athulya*, CR 43–7 and 9S127. It was supported by Table 4 as these were the genotypes classified as stable or fairly stable for all the traits. Besides, among these four, 8S501 had the highest predicted mean (BLUP) for YPP, HI, DM and second-highest for SYP. Therefore, the selection of these genotypes was justified. The selection of these genotypes would greatly benefit the improvement in mean performance as reflected by the high per cent of selection differentials. Selection of these genotypes would result in a genetic gain of 0.60 kg/plant for YPP accounting for 62 % improvement in the trait. Similarly, a genetic gain of 0.14 in HI, 3.53 % in DM and 139 g/plant in SYP would be possible by the selection of these genotypes. Zuffo et al. (2020) also

identified stable soya bean genotypes under drought and salinity situations through MTIS.

5. Conclusion

It could be concluded that the environment had a significant role in affecting the phenotypic expression of the traits in cassava. High GCV, PCV, heritability and GAM would result in effective selection for the improvement of LAI, YPP and SYP. Dry matter content was highly influenced by the environment as evidenced by the high difference between genotype and environment effect and low GAM value suggesting that simple selection may not be fruitful for the improvement of this trait. WAAS biplots captured the entire $G \times E$ interaction. The clones, 9S127 and 8S501 could be recommended for general cultivation as they were identified as stable or fairly stable for the important characters such as LAI, YPP, HI and DM. The rainy and water stress were the two different mega environments which influenced the traits differently. The above average environment mean values of rainy seasons of 2017–18 and 2018–19 for all the traits pointed that they were favoured by the rainy season. The below average environment mean values of water stress of 2018–19 and 2019–20 suggested that water stress reduced the traits performance. The genotype 8S501 had very high BLUP mean values for YPP, HI, DM and SYP depicting that it was most suitable genotype for exploiting these traits. Similarly, MTSI also indicated that 8S501 was the most ideal genotype followed by *Sree Athulya*, CR 43–7 and 9S127 based on YPP, HI, DM and SYP. These new techniques WAASBY and MTSI would be very useful to the plant breeders for the selection of superior genotypes for multiple traits based on multi-environment data.

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CRediT authorship contribution statement

A.V.V. Koundinya: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing - original draft, Writing - review & editing. **B.R. Ajeesh:** Investigation, Writing - original draft, Writing - review & editing. **Vivek Hegde:** Conceptualization, Investigation, Writing - review & editing. **M.N. Sheela:** Project administration, Resources. **C. Mohan:** Project administration, Resources, Writing - review & editing. **K.I. Asha:** Project administration, Resources.

Declaration of Competing Interest

Authors declare no competing interest.

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