

Intra- and Inter-Season Stability of Cassava Plant Morphological Traits Associated with Host-Plant Resistance Against Cassava Green Mite in Zambia

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Abstract: Cassava genotypes that combine earliness with prolonged underground storability are most preferred for food security under subsistence farming. However, the long growth cycle of cassava coupled with the delayed harvesting by local farmers in Zambia exposes the crop to cassava green mite (CGM) attack which contributes to instability in yield performances of cassava. Various plant morphological traits have been recognized as direct or indirect defense mechanisms that enhance host plant resistance (HPR) to CGM. However, little research has been done to understand the stability of such traits despite their potential impact on the durability of HPR. With this background, field trials, involving sequential harvesting of cassava at 9, 12, and 15 months after planting (MAP) were conducted for two seasons. The objective of the study was to understand the variability of the indirect plant defense mechanisms, and how the interactions of genetic factors with crop age and season influence the expression of these vital traits. The genotype stability index was computed for each genotype for CGM population density and leaf damage, leaf retention, stay green, and apical leaf pubescence. There were highly significant differences among genotypes at different sampling dates for all the traits studied. Genotypes TMS 4 (2) 1425, L9.304/175, and L9.304/147 exhibited high intra-season and inter-season stability for low CGM-induced leaf damage. Genotypes Kapeza, Bangweulu and I60/42 exhibited a tolerance mechanism towards CGM. Two of these genotypes L9.304/175, and TMS 4 (2) 1425 also combined high intra-season and inter-season stability for increased leaf retention and apical leaf pubescence, while Kapeza and Bangweulu combined high inter-season stability for increased stay green and leaf retention. Genotypes that combined intra- and inter-season stability for both Low CGM population density and low CGM-induced leaf damage were also identified.

Keywords: *Mononychellus tanajoa*, Host-Plant Resistance, Stability, Intra-Season, Inter-Season

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the second major staple after maize (*Zea mays* L) in Zambia and it serves as a source of livelihood for more than 6 million people. Cassava offers the advantage of flexible harvesting, allowing farmers to keep the storage roots underground until needed [19]. However, the long growth cycle of many cassava genotypes is one of the constraints hampering the

adoption of the crop by young farmers who would otherwise engage in cassava production as a business. In turn this indicates a clear need for early bulking cassava genotypes. Existing improved cultivars in Zambia take 14-16 months to provide reasonable yields, while most landraces take a minimum of 24 months.

Long growing season requirements of cassava and the varying agronomic conditions in which cassava is cultivated expose the crop to numerous biotic and abiotic stresses [3], a combination of which can result in devastating effects on

storage root yield [1]. Seasonal variability of cassava pests and/or disease pressure has been widely reported [29]. During the dry season, combined attack of cassava green mite (CGM) and termites (*Microtermes* sp) coupled with lack of moisture aggravate yield losses [1, 4, 25]. It is also documented that the impact of pest or disease attack varies with the genotype and growth stage at which the injury or damage is caused [24].

Few studies are available on how CGM is influenced by phenotypic traits such as leaf pubescence (Pbs) [11], colour or shape of leaf [12], size and compactness (TC) of shoot apices (TS) [29], leaf retention (LR) and stay green (SG) [18], and environmental factors such as rainfall, temperature, relative humidity [29], and ultra-violet radiation [20]. Research has shown that high Pbs protects natural enemies of CGM, particularly the phytoseiid predatory mite *Typhlodromalus aripo*, against harsh weather conditions, supporting its continuous survival in cassava fields [15, 29]. Studies have shown that pubescent cassava genotypes tend to release volatiles that attract *T. aripo* [21]. However, due to the fact that pubescent genotypes may also differ in other traits that confer resistance to mites, further study is required to determine if leaf hair density is the primary mechanism of resistance of cassava to CGM [16]. Moreover, little research has been done to understand the variability of the aforementioned traits, and how the interactions of genetic factors with crop age and season influence the expression of these vital indirect plant defense mechanisms [6, 29]. It is

envisaged that selecting genotypes for high intra- and inter-season stability of enhanced CGM resistance-conferring traits [8], would in turn enhance the durability of host plant resistance (HPR) [3], and promote biological control by supporting continuous survival of natural enemies in cassava fields planted to improved cultivars [23, 29]. Knowledge of the stability of desirable traits across different selection stages or stages of plant growth would also enable a breeder to more accurately predict the performance of genotypes at later stages in the breeding programme and for release purposes. Therefore, the breeder can make decisions at an early stage of breeding and/or without waiting for the crop to reach full maturity [13]. Against this background, trials were conducted in order to establish the within year (season) and between years stability of genotypes for traits that enhance the resistance of cassava to CGM and the ability of cassava to host *T. aripo*.

2. Materials and Methods

2.1. Study Site

The study was conducted at Kawiko which is located 11°45'E and 24°23'S at 1363 m above sea level in the Mwinilunga district of Zambia. Details of the weather conditions during the 2017/18 and 2018/19 seasons the study was conducted in, and soil nutrient analyses are presented in Table 1.

Table 1. Climatic Data and Soil Nutrient Analysis for Kawiko Agricultural Camp, Mwinilunga, Zambia (2017/2018 and 2018/2019 Seasons).

Year	Climatic Parameters			pH	Soil Chemical Parameters									
	Rainfall (mm)	Temp (°C)	RH (%)		P	Al	Ca	Mg	K	CEC	Zn	Cu	N	C
	Nov-Mar	Min-Max	Mean			Ppm	ppm	%	%					
2018	1374	10-24	72	4.2	6	1.8	0.32	0.83	0.38	11.2	18.7	0.1	0.1	1.7
2019	1200	12-27	75	4.1	3	2.6	0.26	0.85	0.32	11.0	16.2	3.0	0.2	0.64

Temp= temperature in degrees Celsius (°C); Min=Minimum temperature; Max=maximum temperature; RH=average relative humidity measured as a percentage; pH=potential of hydrogen ions as a measure of soil acidity based on calcium chloride; ppm =parts per million; Meq =milli-equivalent.

2.2. Experimental Materials

Nineteen cassava genotypes, which included five landraces, eight locally improved, and six introductions from the International Institute of Tropical Agriculture (IITA) in Nigeria, were evaluated.

2.3. Experimental Design and Layout

The experiment was laid out in a randomized complete block design with three replications. The materials were grown and evaluated over two growing seasons under rain-fed conditions. The first trial was planted on 15th December 2017 and evaluated from January 2018 to March 2019. The same genotypes were planted in the second trial on 15th December 2018 and evaluated from January 2019 to March 2020. Each plot consisted of 36 plants spaced at 1 m between rows and 1 m within rows, equivalent to 10,000 plants ha⁻¹.

2.4. Inoculation of Experimental Materials

The borders of each plot were planted with a CGM susceptible genotype. Two months after planting (in February each year), the borders were artificially infested with CGM from a screenhouse-raised colony by attaching two infested leaves, which had at least 20 adult mites each, onto each of the border plants in every replication [10]. The petiole of each infested leaf was lightly tied with string to the petiole of the first and second fully expanded leaf from the top of each of the two plants per clone. The detached infested leaf and the attached uninfested leaf were placed with their abaxial surfaces in contact with each other. The main lobes were lightly held together with a plastic-coated paper clip leaving the other leaf lobes free. The infester leaves and paper clips were removed after three days. Inoculation was repeated soon after the cold season in August. No fertilizers or herbicides were applied, but the trial was kept weed-free by frequent hand weeding.

2.5. Data Collection

The CGM population density (CGM PD) and CGM leaf damage (CGM LD) were recorded as suggested by [11], using a rating system which involved estimating the proportion of leaf area covered by chlorotic spots, and the counting of adult mites on the third fully expanded leaf from the top on each of six randomly selected plants in each plot. The CGM LD was based on a 1-5 scale, where: 1=no obvious symptoms; 2=moderate damage, no reduction in leaf size, scattered chlorotic spots on young leaves, 1-2 spots cm⁻²; 3=severe chlorotic symptoms, light reduction in leaf size, stunted shoot, 5-10 spots cm⁻²; 4=severe chlorotic symptoms and leaf size of young leaves severely reduced; and 5=tips of affected plants defoliated, resulting in a candle stick appearance of shoot tips. Plants with scores of 1 and 2 were considered to be resistant, whereas plants with scores of 3 to 5 were considered to be susceptible to CGM. Each genotype was scored for the following traits on a 1 to 3 scale: (i) Pbs; where: 1=glabrous, 2=moderately pubescent, and 3=highly pubescent; (ii) TC, where: 1=loose, 2=moderately compact, and 3=compact; (iii) TS, where: 1=small, 2=medium, and 3=large; (iv) leaf longevity assessed by scoring for LR and SG, where for LR: 1=poor (<50% of the leaves retained); 2=good (50-75% of the leaves retained); 3=very good (≥75% of the leaves retained).

2.6. Stability Assessment

Stability assessment was performed using [26] ecovalence stability measure (Wi) using the formula:

$$W_i = \sum (X_{ij} - X_i - X_j + X_{..})^2$$

Where: W_{ij} =ecovalence of the i^{th} genotype, X_{ij} =the observed phenotypic value of the i^{th} genotype in the j^{th} season (or sampling date), X_i =mean of i^{th} genotype across the seasons (or sampling dates), X_j =mean of j^{th} season (or sampling date), $X_{..}$ = grand mean. Genotypes with the lowest W_{ii} value were regarded as the most stable across sampling dates and/or seasons.

Genotype stability index: A stability index was calculated

for each genotype based on combining the ranking of overall mean performances for each trait and the ranking for W_i stability score for each trait. This stability index which is normally applied to yield data and is referred to as yield stability index (YSI) [9] was also applied in this study to mean performances of genotypes for other traits and referred to as genotype stability index (GSI). Instead of using the AMMI stability value as is normally the case for YSI, the GSI was calculated as the sum of ranks for W_i -ecovalence stability index and trait overall mean using the modified formula (after Farshadfar [8]):

$$GSI_i = \sum RW_i + \sum RY_i$$

Where: GSI_i =genotype stability index for the i^{th} genotype across sampling dates or seasons for each trait; $\sum RW_i$ =sum of ranks of the i^{th} genotype across sampling dates within a season or across seasons based on W_i ; $\sum RY_i$ =sum of ranks of the i^{th} genotype based on mean performance across sampling dates (S-date) within a season or across seasons. The genotype with the lowest GSI was considered the best for a particular trait across sampling dates, and a genotype with lowest GSI rank sum over the two seasons was considered the best for a trait across seasons. The W_i was chosen because it is very easy to compute and has no restrictions pertaining to the number of environments as is the case with AMMI stability variance.

3. Results

The ANOVA were performed using Genstat statistical package [22] for each season separately and only combined for sampling dates within each season (Table 2). The means squares (MS) for genotype (G) main effects were significant ($P < 0.05$) for all the traits measured across sampling dates (S-dates) and seasons. The S-date MS were significant for all the traits measured in the 2017/18 season. The G x S-date MS were also significant for CGM LD, SG in the 2017/18 season. However, the G x S-date MS were not significant for any of the traits measured in the 2018/19 season.

Table 2. Analysis of 19 Cassava Genotypes Evaluated for Resistance to Green Mite Density and Associated Leaf Damage, Leaf Retention, Stay Green, and Leaf Pubescence at Three Sampling Dates in Zambia.

Source of variation	df	Mean Squares				
		CGM PD	CGM LD	LR	SG	Pbs
2017/18						
Genotype (G)	18	2101.9***	1.4***	433.7***	3.0***	2.2***
Sampling date (S)	2	1472.4**	1.0*	2993.4***	2.0***	1.1
G x S	36	173.1	0.5*	501.6***	0.6*	0.2
Residual	112	215.5	0.3	163.1	0.4	0.3
2018/19						
Genotype (G)	18	2227.9***	1.5***	709.0***	3.4	1.7***
Sampling date (S)	2	499.1	0.1	856.6*	0.6	0.2
G x S	36	120.6	0.2	106.6	0.2	0.2
Residual	112	349.4	0.4	252.0	0.4	0.5

CGM PD=population counts of cassava green mites per leaf; CGM LD=level of leaf injury caused by cassava green mite; LR=proportion of leaves retained on a plant; SG=stay green; Pbs=pubescence of apical leaves * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ 4.3.1.

Genotypes which had lowest rank sum for Wi across the sampling dates for a particular trait were considered to exhibit high intra-season stability, while genotypes which had lowest rank sum for Wi across season were considered to exhibit high inter-season stability. Genotypes with lowest GSI scores combine high stability with desirable trait means and were therefore considered to be the most stable and superior for the trait, while genotypes with high GSI scores are undesirable.

Cassava green mite population density: Genotypes TMS 4 (2) 1425 and L9.304/147 had the lowest GSI overall for CGM PD and so were the most resistant and most stable across

sampling dates in each of the seasons and across seasons (Table 3).

Cassava green mite leaf damage: Genotypes Kapeza and Bangweulu had lowest GSI for CGM LD across sampling dates in the 2017/18 season. Genotypes TMS 4 (2) 1425, Bangweulu and L9.304/175 had lowest GSI for CGM LD across sampling dates in 2018/19. Overall, genotypes TMS 4 (2) 1425, Bangweulu, and I60/42 were the most stable and most resistant across seasons, while Mweru and Kariba were the most susceptible and least stable genotypes across seasons (Table 3).

Table 3. Ranked intra- and Inter-Season Stability Indices for Cassava Green Mite Population Density and Associated Leaf Damage of 19 Cassava Genotypes Evaluated in 2017/18 and 2018/19 Seasons at KAWIKO in mwinilunga District, Zambia.

Genotype	CGM PD						CGM LD					
	2017/18		2018/19		Overall		2017/18		2018/19		Overall	
	GSI	Rank										
Kapeza	18	8	28	15	46	14	7	1	15	5	71	5
Mweru	25	14	37	19	62	18	36	19	19	19	31	19
M86/0016	32	18	20	12	52	16	20	12	15	13	44	13
L9.304/147	7	2	6	1	13	1	13	5	9	5	31	5
Bangweulu	23	11	16	7	39	7	10	2	2	2	24	2
Chila	25	14	22	13	47	15	24	14	14	15	46	16
Lelanyana	22	10	35	18	57	17	16	8	9	9	34	9
I60/42	24	12	16	7	40	9	11	3	4	3	26	3
Lufunda	30	16	11	3	41	10	16	8	4	5	31	5
I30040	12	4	17	9	29	5	14	6	11	9	34	9
L9.304/175	10	3	19	10	29	5	16	6	2	4	30	4
14 (2) 1425	4	1	11	3	15	2	12	4	1	1	18	1
Manyopola	13	6	30	16	43	12	24	14	17	17	49	17
Kampolombo	13	6	26	14	35	7	25	16	4	12	40	5
92/0000	19	9	9	2	28	4	14	6	7	5	31	12
L9.304/36	30	16	11	3	41	10	29	17	7	15	46	15
Kariba	22	19	31	17	64	19	34	18	18	18	60	18
TME 2	24	12	19	10	43	12	23	13	12	13	44	13
Kaleleki	19	4	15	6	27	3	16	8	12	11	37	11

CGM PD=cassava green mite population density per leaf; CGM LD=cassava green mite leaf damage scored on 1-5 scale, where 1=no damage, and 5=very severe damage; GSI=genotype stability index

Leaf Retention: In the 2017/18 season, Bangweulu, Kapeza and Mweru combined high stability with highest LR, while Kampolombo, L9.304/36, and L9.304/147 combined low stability with low LR across sampling dates (Table 4). In the 2018/19 season, smallest GSI scores for LR were recorded for genotypes TMS 4 (2) 1425, L9.304/147, and Kariba. Genotypes TMS 4 (2) 1425 and Kapeza exhibited high stability for LR combined with high mean for the trait across the two seasons, while Kampolombo and L9.304/36 combined low stability with low LR (Table 4).

Stay Green: Kampolombo, 92/000 and Bangweulu had lowest GSI scores for SG, while Manyopola, I60/42 and Kariba combined low stability with low SG in the 2017/18 season (Table 4). In the 2018/19 season, 92/000 and Bangweulu had the lowest GSI for SG, while M86/0016, Lufunda and I60/42 combined low stability with lowest means for SG across sampling dates in the season. Overall, 92/000

and Kampolombo had the lowest GSI scores and were therefore the most stable with high SG across seasons.

Leaf Pubescence: Genotypes Kaleleki, L9.304/175, and 92/000 had the lowest GSI for Pbs and were therefore considered to be the most stable and the most pubescent across sampling dates in 2017/18 season, while Chila, Kariba and Bangweulu had the highest GSI scores and were considered to be the least stable and least pubescent genotypes across sampling dates in the season (Table 4). Genotypes TMS 4 (2) 1425, L9.304/147 and L9.304/175 were the most stable and the most pubescent across sampling dates in 2018/19 season (Table 3). Overall, L9.304/147 and TMS 4 (2) 1425 had lowest GSI scores and were therefore the most stable and the most pubescent genotypes across the two seasons, while largest GSI scores were recorded for I60/42, Kampolombo, and M86/0016 which were therefore considered to be the least stable and the least pubescent genotypes across the seasons

(Table 5).

Table 4. Ranked Intra-Season Stability Indices for Leaf Retention, Stay Green, and Leaf Pubescence of 19 Cassava Genotypes Evaluated Across Three Sampling Dates in 2017/18 and 2018/19 Seasons.

Genotype	Leaf Retention				Stay Green				Pubescence			
	2017/18		2018/19		2017/18		2018/19		2017/18		2018/19	
	GSI	Rank	GSI	Rank	GSI	Rank	GSI	Rank	GSI	Rank	GSI	Rank
Kapeza	6	2	19	8	17	4	13	5	10	4	26	14
Mweru	7	3	26	16	22	12	28	15	24	15	15	6
M86/0016	20	7	20	10	18	16	36	19	22	12	31	18
L9.304/147	29	17	11	2	14	6	22	12	20	11	5	2
Bangweulu	4	1	22	13	18	3	7	1	27	17	22	12
Chila	10	4	19	8	18	6	15	7	32	19	16	7
Lelanyana	20	7	21	11	31	6	17	8	25	16	20	11
I60/42	25	13	21	11	23	19	29	17	22	12	34	19
Lufunda	20	7	35	18	20	14	29	17	18	7	24	13
I30040	25	13	28	17	20	10	21	10	19	9	19	9
L9.304/175	15	5	18	7	26	10	20	9	6	2	7	3
14 (2) 1425	21	11	4	1	26	15	13	5	15	5	3	1
Manyopola	20	7	14	6	29	18	25	14	19	9	28	15
Kampolombo	30	18	26	19	7	2	8	3	22	12	29	17
92/0000	25	13	13	4	5	1	7	1	9	3	11	4
L9.304/36	31	19	25	15	19	9	27	10	17	6	28	15
Kariba	26	16	12	3	28	16	28	15	27	17	19	9
TME 2	21	11	13	4	17	4	24	13	18	7	11	4
Kaleleki	18	6	23	14	22	12	11	4	3	1	16	7

LR=leaf retention expressed as a percentage; SG= stay green scored on 1-3 scale, where 1=lowest, and 3=highest; Pbs=leaf pubescence score on 1-3 scale, where 1=glabrous, and 3=highly pubescent; GSI=genotype stability index

Table 5. Ranked Inter-Season Stability Indices for Leaf Retention, Stay Green, and Leaf Pubescence of 19 Cassava Genotypes Evaluated Across 2017/2018 and 2018/2019 Seasons.

Genotype	Leaf Retention		Stay Green		Pubescence	
	GSI	Rank	GSI	Rank	GSI	Rank
Kapeza	25	1	30	4	36	7
Mweru	33	5	50	14	39	9
M86/0016	40	11	64	19	53	18
L9.304/147	40	11	40	9	25	5
Bangweulu	26	3	21	3	49	16
Chila	29	4	33	5	48	15
Lelanyana	41	13	35	7	45	11
I60/42	46	15	60	18	56	19
Lufunda	55	17	52	15	42	10
I30040	53	16	41	12	38	8
L9.304/175	33	5	40	9	13	1
14 (2) 1425	25	1	39	8	18	2
Manyopola	34	7	54	16	47	14
Kampolombo	66	19	15	2	51	17
92/0000	38	9	12	1	20	4
L9.304/36	56	18	40	9	45	11
Kariba	38	9	56	17	46	13
TME 2	34	7	41	12	29	6
Kaleleki	41	13	33	5	19	3

4. Discussion

The study has clearly indicated effects of seasonal variations on the performance and stability of cassava genotypes. The average daily temperatures of 28°C and relative humidity of 72-75% experienced during the seasons seem to coincide with the optimum temperature of 27°C and RH of 50-70% reported for maximum oviposition of CGM [11, 27]. This is a probable reason for the highest CGM PD recorded at 9 MAP. Heavy rains are normally experienced in

December (second sampling) while March (third sampling) coincides with the end of the rainy season. Consistent with this observation, the author [27] attributed increased CGM mortality to the mites being washed off the leaves during the wet season. The minimum temperature of 10°C experienced in June and July, which happened to be lower than the estimated thermal threshold for CGM of 14.4°C [28], is another source of mite mortality [15].

Locally improved genotype L9.304/147 exhibited better levels of stability for low CGM PD as compared to TMS 4 (2) 1425 and I60/42, which are widely used as sources of

resistance to CGM in Africa [11, 14]. Similarly, the high stability for low CGM PD as displayed by a landrace Kaleleki indicates that locally adapted sources of resistance are available. Having been grown in the locality for several years, landraces are more likely to cope with environmental stresses including crop pests and diseases common to a given locality, making them suitable candidates for inclusion as parents in a breeding programme [24].

Genotypes Kapeza and I60/42 were better ranked for CGM LD than they were for CGM PD, which suggests that, these genotypes exhibit a tolerance mechanism towards CGM. Consequently, genotypes which combine low CGM PD with low CGM LD, such as TMS 4 (2) 1425, L9.304/147, and L9.304/175, are the most desirable and can be recommended for wider production, or as sources of resistance for breeding programmes.

The study revealed the presence of genetic variability in the germplasm for LR in Zambia. Six genotypes that combined high stability with high mean LR had one characteristic in common, namely a tendency to either fold or roll their leaves downward away from the sun during hot periods. According to the research [7], the action of leaf folding may be a mechanism for water stress avoidance. It is also suggested that genotypes which exhibit high LR combined with enhanced SG are likely to be resistant to both CGM and drought [18].

In cassava, Pbs is said to be the primary trait responsible for resistance to CGM [24]. The Pbs, especially of immature leaves and shoot apices, has been reported to provide suitable habitat for *T. aripo* which has proved to be the most successful natural enemy against *M. tanajoa* and whitefly (*Bemisia tabaci* Gennadius) in Africa [2, 20]. The current study was conducted in the absence of the natural enemy, and therefore, it was not possible to confirm or otherwise these reports, but this study indicated that the trait is little influenced by seasonal effects and that there is genetic variability for this in the local Zambian germplasm. The results of the current study coupled with other reports of heritability estimates as high as 93% for this trait [11], imply that the expression of Pbs is highly predictable and therefore it should be relatively easy to incorporate into new genotypes [5]. Three genotypes which exhibited the highest stability combined with high level of Pbs were L9.304/175, TMS 4 (2) 1425, and Kaleleki. These genotypes had high inter-season stability for low CGM PD and CGM LD, and could be used as sources of genes for CGM resistance.

5. Conclusion

Overall, the study has shown that there is wide diversity in the expression of valuable indirect defense traits among genotype, indicating that there is scope for integration of biological control and host plant resistance for CGM in Zambia. Release of genotypes that exhibit high levels of intra-season and inter-season stability for enhanced expression of LR, SG, and Pbs will minimize the impact of CGM on storage root yield and root dry matter content of

cassava that results from seasonal effects in Zambia. The study has identified genotypes which have good stability across seasons within a year and across years. Such genotypes will also provide the required habitat for *T. aripo* in cassava fields.

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