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DECODING ANTIXENOTIC MECHANISM OF RESISTANCE IN CASSAVA GENOTYPES AGAINST WHITEFLY, *BEMISIA TABACI* (GENNADIUS)

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ABSTRACT

Screening 375 cassava genotypes at Tapioca and Castor Research Station, Salem, India against whitefly, *Bemisia tabaci* (Gennadius) revealed eight highly resistant genotypes namely, Me 743, Me 650, Me 637, Me 739, Me 148, Me 874, Me 25, and Me 707, maintaining fewer than 20 insects/ plant across crucial growth stages (3-6 months after planting). Trichome analysis revealed prevalent non-glandular, simple, single-celled elongated or irregular-shaped structures. Correlation studies between whitefly incidence and trichome density indicated a positive relationship (0.39) without statistical significance, emphasizing the role of trichomes in conferring resistance. India's cassava genetic diversity holds significant promise in the creation of resistant variety.

Key words: Cassava, *Manihot esculenta*, germplasm, *Bemisia tabaci*, virus - vector, cassava mosaic virus, resistance, field screening, population count, trichomes types, scanning electron microscopy, density, correlation studies

The global impact of whitefly, *Bemisia tabaci* (Gennadius) has intensified due to its swift proliferation across tropical and subtropical regions. In India, it poses a significant threat to cassava by spreading Cassava Mosaic Begomovirus (CMB) and causes substantial damage to cassava crops by feeding on their sap, secreting a sugary substance that fosters sooty mould growth, hindering plant respiration and photosynthesis (Nelson, 2008) and also acts as a carrier for various plant viral infections (Brown and Czosnek, 2002; Basu, 2019). Cassava (*Manihot esculenta* Crantz) serves as a vital nutritional source for over a billion people across 105 countries, yielding around 302 million tonnes annually (Latif and Müller, 2015; FAOSTAT, 2022). In Tamil Nadu, contributed 83.75% of India's total output in 2021-22 (APEDA, 2022). In the early 1940s, the discovery of the Cassava Mosaic Virus (CMV) posed a severe risk to cassava in India (Abraham, 1956). Indian Cassava Mosaic Virus (ICMV) and Sri Lankan Cassava Mosaic Virus (SLCMV) transmitted by *B. tabaci* through semi persistent manner and, are prevalent in the India (Varma et al., 2011). Following *B. tabaci* infection, CMD symptoms in cassava typically appear about a month after the first leaves emerge, peaking around 60 days post-planting. Infections introduced beyond five months after planting have minimal impact on yield since tuber formation begins by that time, allowing the plant to sustain significant yield (Fargette et al., 1990). According to Legg et al. (2011), a count of over five adults per top five leaves per plant was categorized as highly abundant, whereas Omongo et al. (2012) classified populations as high only when surpassing 20 adults per top five leaves per plant. Managing whitefly populations through insecticides poses challenges due to their rapid resistance development and the associated costs (Basit, 2019; Horowitz et al., 2020). Host plant resistance emerges as a more suitable and sustainable approach for effectively managing whitefly infestations.

Plant trichomes have significant influence on *B. tabaci* interaction with plants. Trichome characteristics, particularly density and the presence of glandular exudates, significantly influence the host plant selection process of adult whiteflies for oviposition (Avery et al., 2015). Oriani et al. (2010) and Firdaus et al. (2011) uncovered a significant link between plant trichomes and *B. tabaci* behavior. Presence on upper or under surface of laves play a pivotal role in influencing *B. tabaci* interactions with their host plants. This study's focus on evaluating cassava genotypes for *B. tabaci* resistance and understanding how trichomes influence whitefly preference lays a crucial foundation for future

endeavors. The insights gained pave the way for targeted breeding programs aimed at developing *B. tabaci* resistant cassava varieties, offering promising strategies for effectively managing *B. tabaci* infestations in cassava crops.

MATERIALS AND METHODS

A total of 375 distinct available cassava genotypes were selected for whitefly resistance screening from Tapioca and Castor Research Station (TCRS), Yethapur, Salem, Tamil Nadu, India which is a center for collection, conservation, cataloguing and evaluation of genetic resource of cassava under All India Co-ordinated Research Program (AICRP) on Tuber crops. In 2022-2023, 375 genotypes were screened at Field No. B1, B2 and B3 with the area of one acre located at Tapioca and Caster Research Station (TCRS), Yethapur, Salem. Fifteen cassava cuttings of each genotype were planted in each row with the plant to plant and row to row spacing of 90x90 cm^2. The ploughed and harrowed field was lined and pegged before planting. The experiment was set out under drip irrigation conditions and weeding was done manually using a hoe or cutlass when necessary. To assess resistance against *B. tabaci*, adult counts were conducted on abaxial surface of the top 5 leaves of five randomly chosen plants from each genotype for four consecutive periods (82, 112, 142 and 172 DAP) as per Omongo et al. (2012). The data collected from five plants within each genotype were summarized, and mean values were derived. Analysis of variance (ANOVA) was employed to analyze the means, followed by a Tukey test for multiple comparisons using R software.

Out of 375 cassava genotypes, 50 were specifically chosen for the trichome study, ensuring representation from both high and low populations of whiteflies. In this study, fully grown leaves from 3 to 4 month old plants, coinciding with high insect populations, were examined. The investigation focused on selected cassava genotypes, analyzing the presence or absence of trichomes on the abaxial leaf surface epidermis and veins. Trichome density within a 4 mm^2 leaf area was assessed across each genotype, utilizing a Leica WILD M10 stereo zoom microscope. The study involved measuring trichome type, length, and spacing, aiming to understand their characteristics. Further, a correlation matrix between trichomes and whitefly population was established using R software (Pastório et al., 2022). For scanning electron microscope (SEM) analysis 1 mm^2 area from the central leaf lobe's median section of each cassava cultivar was carefully excised using a scalpel. Before SEM imaging these samples were processed according to Pastório et al. (2019).

RESULTS AND DISCUSSION

The assessment of whitefly population dynamics during cropping periods unveiled significant fluctuations, notably peaking at 112 days after planting (DAP) - range from 4 to 131 (Fig. 1) and averaging 5.21, 55.17, 24.70 and 15.28 insects/ plant at 82, 112, 142 and 172 DAP (Table 1). These findings are similar with those of Gwandu et al. (2019) study, which identified highest nymphal counts and whitefly induced damage during the 4th and 5th months after planting (MAP). These results emphasizing the need for targeted interventions during 4–5 MAP to mitigate yield losses. Screening of 375 genotypes highlighted eight specific accessions including Me 743, Me 650, Me 637, Me 739, Me 148, Me 874, Me 25, and Me 707 that consistently displayed high resistance. Omongo et al. (2012) gave a classification criteria for whitefly resistance and they identified that South American genotype MEcu 72 and several Ugandan cassava landraces were highly resistance, Gwandu et al. (2019) identified resistance based on nymphal count and adult damage and identified ten genotypes. Barilli et al. (2019) in Brazil identified resistant genotypes, MEcu 72 and Santa Helena against *B. tuberculata*. The cassava genotypes namely Me 819, Me 834, Me 792, Me 460, Me 812, and Me 827 showed high susceptibility hosting over 100 insects by 112 DAP, averaging more than 45 insects across all observation periods.

In exploring cassava trichomes through stereo and

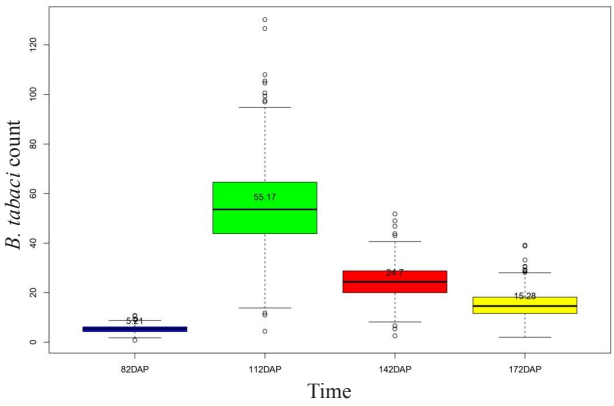

Fig. 1. *Bemisia tabaci* – population dynamics

Population dynamicsacross time periods represented using box plot. X axis represents time periods 82 DAP, 112 DAP, 142 DAP and 172 DAP (days after planting). Y axis represents counts (numbers/ plant).

Table 1. *B. tabaci* incidence across genotypes and time periods

Time period	Source	Df	Mean counts	Sum sq	Mean sq	F value	Pr (>F)
82 DAP	Genotypes	374	5.21	3882	10.381	7.099	$< 2e^{-16}$ ***
	Replication	4		73	18.145	12.409	$6.23e^{-10}$ ***
112 DAP	Genotypes	374	55.17	595001	1590.9	10.598	$< 2e^{-16}$ ***
	Replication	4		4509	1127.2	7.509	$5.48e^{-06}$ ***
142 DAP	Genotypes	374	24.69	91102	243.6	10.70	$< 2e^{-16}$ ***
	Replication	4		1640	410.0	18.01	$1.86e^{-14}$ ***
172 DAP	Genotypes	374	15.27	56856	152.02	9.672	$< 2e^{-16}$ ***
	Replication	4		918	229.56	14.605	$1.05e^{-11}$ ***

This table compared the "genotypes" and "replication" factors for each time period (82, 112, 142, and 172 DAP). The table helps compare the ANOVA results between time periods focusing on the key statistics. Signif. codes: 0 '***' 0.001 '**' 0.01 '*'.

scanning electron microscopy, trichome present in cassava were observed to be simple, single-celled type, often elongated or irregular in shape, lacking glandular characteristics. Out of 50 selected genotypes with ranges of whitefly population, 29 genotypes trichomes entirely (Me 25, Me 75, Me 77, Me 86, Me 131, Me 133, Me 148, Me 149, Me 166, Me 178, Me 179, Me 201, Me 242, Me 243, Me 253, Me 254, Me 256, Me 312, Me 313, Me 330, Me 332, Me 333, Me 343, Me 395, Me 406, Me 454, Me 557, Me 569 and Me 810), while 21 genotypes exhibited these features and among those Me 776, Me 349, Me 168, Me 250, Me 306, and Me 309 showed *B. tabaci* counts of 30.25, 26.75, 32, 29.25, 23, and 26, respectively revealing notably high trichome densities, surpassing 50/ 4 mm² or >12/ mm². These dense concentrations were primarily observed in the midrib and petiole regions, with relatively fewer occurrences in the veins, venules and other leaf area. Marin et al. (2020) while studying trichomes of ten cassava varieties in Colombia got similar results. The length and width of the trichome ranges from 100-500 µm and 5-10 µm, respectively, and the gap between them ranges from 0 - 150 µm in midribs and 50 - 200 µm in veins, venules and other leaf area (Fig. 2). The correlation between trichome density and *B. tabaci* counts showed a positive correlation (0.39) without statistical significance. Previous studies, by Pastório et al. (2022), reported the non-glandular nature of cassava trichomes and showed higher *B. tuberculata* nymph abundance in cultivars with denser trichomes on sprout and apical leaves. Studies on cotton, tomato, potato, pepper and brinjal also revealed increased *B. tabaci* oviposition to highly pubescent leaves (Oriani et al., 2010; Firdaus et al., 2011; do Prado et al., 2016; Hasanuzzaman et al., 2016). However, Valle et al. (2012), found no significant correlation between trichome density, oviposition preference, and adult attractiveness. Eight genotypes identified in this study appear promising as potential parent material for breeding resistant cassava varieties.

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AUTHOR CONTRIBUTION STATEMENT

KV conducted all the field experiments and manuscript drafting. SJ, MM and KS conceived the hypothesis, designed the experiment and corrected the manuscript. PK and RV assisted in conducting field experiment. PK assisted in statistical analysis. All authors have seen and approved the manuscript and its contents.

CONFLICT OF INTEREST

No conflict of interest.

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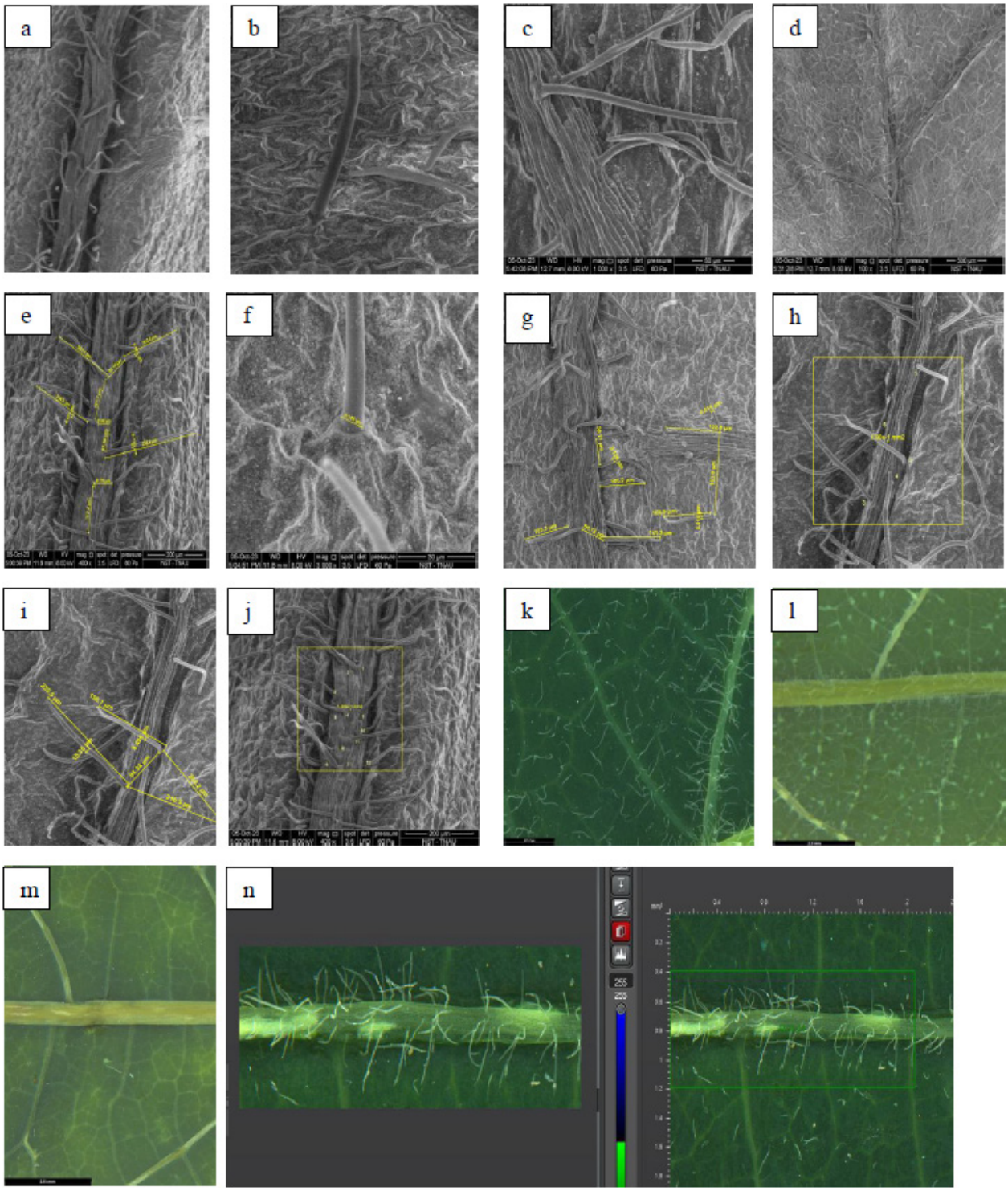

Fig. 2. Trichome characterization: SEM images a-j and stereo zoom microscope images k-n. (a) dense of trichomes at midrib; (b) single celled straight non glandular trichome with sharp end; (c) irregular shared trichomes; (d) trichomes at abaxial surface of leaves; (e, f, g, i) measurement of trichome length, width, distance; (h, j) count of trichomes at 1 mm^2 area; (k) trichomes at veins and venules; (l) dense trichomes at midribs; (m) trichome free leaf surface and a (n) trichome count at midrib

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