Green Canopy Cover via VARI as a Selection Tool for Stay-Green Maize Hybrid

Guilherme Gonçalves Coswosk [1], Valter Jário de Lima [2], Vivane Mirian Lanhellas Gonçalves [3], Messias Gonzaga Pereira [3], Samuel Henrique Kamphorst [3], Eliemar Campostrini [3]

[1] Instituto Federal do Espírito Santo, IFES – Vitória–ES, Brazil – guilhermegc@ifes.edu.br
[2] Universidade Estadual Vale do Acaraú, UVA – São Benedito–CE, Brazil – valter_jario@hotmail.com
[3] Universidade Estadual do Norte Fluminense Darcy Ribeiro, UENF – Campos dos Goytacazes–RJ, Brazil – vivane-lanhellas@hotmail.com, messias@uenf.br, samuelkampho@hotmail.com, campostrini@uenf.br

Keywords: geoprocessing, remote sensing, high-throughput phenotyping, applied statistics, precision agriculture.

1. Introduction

The stay-green trait in maize has become a key target in breeding programs aiming to enhance grain yield (GY) stability under adverse environmental conditions. Characterized by delayed leaf senescence during grain filling, the stay-green phenotype maintains photosynthetic activity and canopy integrity longer, increasing radiation interception and biomass partitioning to kernels. In tropical environments, where high temperature and erratic rainfall are frequent, the ability of a hybrid to maintain a green canopy during reproductive stages is particularly advantageous (Cerrudo et al., 2017).
Remote sensing technologies, especially those based on unmanned aerial vehicles (UAVs), provide a rapid, non-destructive, and high-throughput means to quantify canopy dynamics. Vegetation indices (VIs) derived from RGB images, such as the Visible Atmospherically Resistant Index (VARI), offer a cost-effective alternative to multispectral methods. The VARI index has shown promising results in distinguishing green vegetation from soil background, especially under field conditions where spectral contrast may be subtle (Buchaillot et al., 2019).
This study aimed to quantify green canopy cover (GCC) of maize hybrids using VARI, across different phenological stages, and to investigate its relationship with GY. The objective was to evaluate whether VARI-based green cover could serve as an effective phenotyping tool for selecting high-performing stay-green hybrids adapted to subtropical low-input systems.

2. Materials and Methods

The experiment was conducted at the Experimental Station Ilha Barra do Pomba, Itaocara–RJ (Figure 1). Eight maize hybrids were used: the interpopulation hybrids UENF 506-11 (H1) and UENF 506-16 (H2), both grain-type; UENF MSV 2210 (H3), suitable for silage and green corn; and UENF MS 2208 (H4), for silage. Commercial hybrids included BM 207 (H5) and AG 1051 (H8) (double hybrids); and LG 6036 (H6) and 30F35R (H7) (single hybrids). Due to replanting, H5 was excluded from statistical analyses.
The soil at the experimental site is classified as *Gleissolos Melânico*, characterized by a histic H horizon with less than 40 cm of thickness or by a humic, prominent, or chernozemic A horizon. The soil was fertilized and irrigated according to the recommended crop management practices for the region (van Raij et al., 1997), and cultural treatments were carried out following standard guidelines for maize. Throughout the experimental period, the mean values of photosynthetically active radiation, air temperature, relative humidity, and total precipitation were 1423.6 ± 296.6 µmol m−2 s−1, 22.0 ± 2.9 °C, 75 ± 5.5%, and 100.8 ± 3.3 mm, respectively. Climatological values for each day after planting (DAP) are provided in Figure S1 of the Supplementary Materials, obtained from the Brazilian National Institute of Meteorology (INMET), automatic station no. 604 (Cambuci—RJ), located approximately 12.7 km from the experimental site. To ensure data reliability and quality, records were checked for integrity and consistency through cross-validation with nearby weather stations and historical climatological data.

Figure 1: Schematic diagram of the research area location.

Source: The authors.

The design was a randomized complete block with four replicates (32 plots). Each plot had four 4-m rows (0.70 m between rows; 0.20 m between plants), totalling 80 plants (Gonçalves et al., 2025).
A DJI Mavic 2 Pro UAV, equipped with a Hasselblad L1D-20c 1″ CMOS RGB sensor (20 MP), was used to acquire aerial images at 80 m (2 cm GSD). While the manufacturer provides detailed information on the optical system, public documentation does not include spectral response curves or official per-band wavelength limits, which are proprietary. For descriptive purposes, we therefore report the typical ranges adopted in UAV studies with RGB sensors: Blue ~400–500 nm (0.40–0.50 µm), Green ~500–600 nm (0.50–0.60 µm), and Red ~600–700 nm (0.60–0.70 µm). Flights were carried out at 0 (emergence), 19 (V2–V3), 34 (V3–V4), 91 (R2), 98 (R3–R4), 112 (R4–R5), and 119 (R5–R6). Figure 2 shows the orthophoto captured at 119 DAP.

Figure 2: Orthophoto of the experiment at 119 DAP (R5–R6).

Source: The authors.

VARI was calculated as (G−R)/(G+R+B). In QGIS, thresholding (≥0.02) defined green vegetation. Zonal Statistics was used to extract green canopy cover (%) per plot. GY (13% moisture) was converted to kg/ha (Gonçalves et al., 2025). ANOVA and Tukey tests identified differences; Pearson correlation assessed GCC-yield relationships. All analyses were performed in GENES.

3. Results and Discussion

Table 1 presents the ANOVA results for green canopy cover (GCC) across different phenological stages. Significant differences among hybrids ($p < 0.05$) were observed at 19, 98, 112, and 119 DAP. At 19 DAP (V2–V3), early-stage differences reflected genotypic variation in seedling vigor, which is fundamental for biomass accumulation and initial competition with weeds (Gonçalves et al., 2025).

Table 1: Summary of the analysis of variance for green canopy cover.

DAP	Treat.	Mean	CV%
19 DAP (V2–V3)	0,017503*	0,14	50,03
34 DAP (V3–V4)	0,02778ns	0,73	15,06
91 DAP (R2)	0,000158ns	0,99	1,75
98 DAP (R3–R4)	0,014168*	0,88	7,22
112 DAP (R4–R5)	0,121721*	0,50	37,42
119 DAP (R5–R6)	0,115102**	0,31	46,82

ns — not significant according to the F-test; ** and * significant at 1% and 5% probability levels, respectively, based on the F-test. Treatments (Treat.); Mean (Mean); Coefficient of Variation (CV%); Days After Planting (DAP). Source: The authors.

At 98 DAP (R3–R4), corresponding to the grain formation phase, differences among hybrids in green canopy coverage became more evident. Hybrids LG 6036 (H6) and 30F35R (H7) maintained higher GCC values, suggesting greater leaf longevity and photosynthetic capacity. In contrast, interpopulation UENF hybrids (especially H3 and H4) showed reduced green cover, associated with faster onset of senescence.

At 112 and 119 DAP (R4–R6), these differences were accentuated. As shown in Table 2 (Tukey test), H6 and H7 reached the highest GCC values (~50%), indicating superior stay-green behavior, while H3 and H4 presented values below 15%, characterizing rapid senescence. This phenotypic divergence in canopy maintenance reflects physiological differences in resource allocation and stress resilience (Zheng et al., 2023).

Table 2: Tukey test for comparison of green canopy cover means without height clipping.

DAP	H1	H2	H3	H4	H6	H7	H8
19	0,15 a	0,10 a	0,21 a	0,19 a	0,57 a	0,20 a	0,06 a
34	0,72 a	0,70 a	0,85 a	0,70 a	0,65 a	0,83 a	0,64 a
91	0,99 a	0,99 a	0,98 a	0,98 a	0,99 a	1,00 a	0,98 a
98	0,87 ab	0,89 ab	0,78 b	0,87 ab	0,94 a	0,96 a	0,87 ab
112	0,39 a	0,48 a	0,29 a	0,33 a	0,72 a	0,66 a	0,65 a
119	0,21 ab	0,24 ab	0,14 b	0,12 b	0,50 a	0,52 a	0,41 ab
GY	5452 bc	5675 bc	5420 bc	4900 c	7457 ab	8795 a	7106 ab

Means followed by the same letter in the row do not differ statistically at the 5% probability level, according to Tukey's test. From left to right: H1, H2, H3, H4 (UENF), H6, H7, and H8 (commercial). Days After Planting (DAP). Source: The authors.

The strong positive phenotypic correlations between GCC and grain yield at 112 and 119 DAP ($r = 0.88$ and 0.96, respectively; Table 3) confirm that prolonged canopy greenness enhances sink filling and yield. These results demonstrates that extended photosynthetic duration during grain filling contributes significantly to productivity (Peroni et al., 2020).

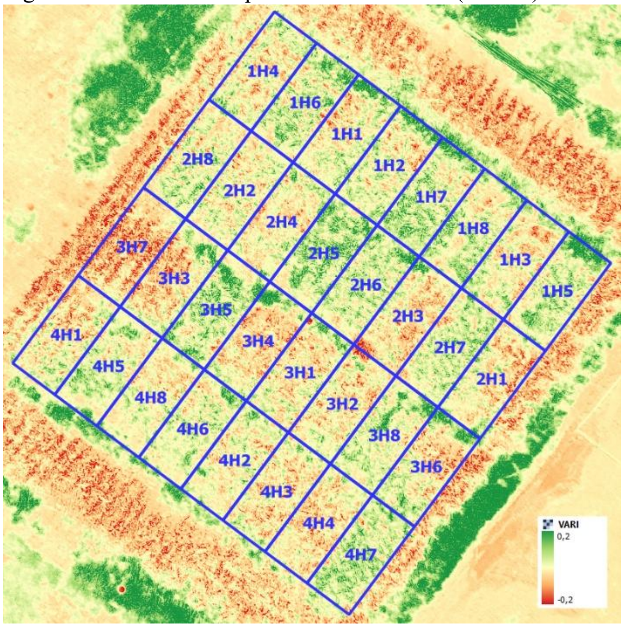
Table 3: Phenotypic correlation between green canopy cover without height clipping and grain yield.

Var.	GCC 19 DAP	GCC 34 DAP	GCC 91 DAP	GCC 98 DAP	GCC 112 DAP	GCC 119 DAP
GY	-0,20	0,06	0,55	0,72	0,88**	0,96**

** and * significant at 1% and 5% probability levels, respectively, based on phenotypic correlation. Days After Planting (DAP). Green Canopy Cover (GCC). Grain Yield (GY). Variable (Var.). Source: The authors.

As illustrated in Figure 3 (VARI map at 119 DAP), commercial hybrids maintained greener canopies longer, which contributes to prolonged photosynthesis and improved yield potential under subtropical conditions (Ribeiro et al., 2021).

Figure 3: VARI of the experiment at 119 DAP (R5–R6).



Source: The authors.

Additionally, your thesis evidences that commercial hybrids with longer reproductive phases preserved canopy integrity longer than UENF hybrids, which have extended vegetative and shorter reproductive stages. This cycle mismatch contributed to early senescence and reduced grain yield in UENF materials (Gonçalves et al., 2025).

Therefore, using VARI for monitoring GCC over time enables effective identification of stay-green genotypes, especially in climates prone to water stress and thermal fluctuations. Integrating such phenotyping tools into breeding programs supports the selection of hybrids with enhanced adaptability and yield stability under subtropical conditions (Gracia-Romero et al., 2019).

Although the experiment was conducted at a single site and on a relatively small scale, measures were taken to minimize microenvironmental variability, including standardized irrigation management and the use of border rows to reduce edge effects. Therefore, the differences observed among hybrids are more likely associated with their intrinsic variability rather than plot position or local heterogeneity. Nevertheless, we acknowledge that testing across multiple environments and larger experimental scales will be essential to strengthen the generalizability of the findings and confirm the robustness of VARI-derived canopy cover as a phenotyping tool for stay-green selection.

4. Conclusion

VARI-derived green canopy cover successfully differentiated maize hybrids with stay-green traits. Commercial hybrids such as LG 6036 and 30F35R maintained higher GCC at late stages and produced greater yields. The VARI index, applied via UAV-RGB imagery, proved to be a reliable tool for high-throughput phenotyping in breeding programs.

5. References

Buchaillot, M.L., Gracia-Romero, A., Vergara-Diaz, O., Zaman-Allah, M.A., Tarekegne, A., Cairns, J.E., Prasanna, B.M., Araus, J.L., Kefauver, S.C. (2019) **Evaluating maize genotype performance under low nitrogen conditions using RGB UAV phenotyping techniques**. Sensors 19:1815. DOI doi.org/10.3390/s19081815.

Cerrudo, D., González, L., Lugo, J.A.M., Trachsel, S. (2017) **Stay-green and associated vegetative indices to breed maize adapted to heat and combined heat-drought stresses**. Remote Sensing 9:235. DOI doi.org/10.3390/rs9030235.

Gonçalves, V.M.L., Crevelari, J.A., Santa Catarina, R., Souza, Y.P., Pereira, M.G. (2025) **Adaptability and stability analysis via GGE biplot in single, double, and interpopulation maize hybrids**. Scientific Reports 15:5065. DOI doi.org/10.1038/s41598-025-89416-z.

Gracia-Romero, A., Kefauver, S.C., Fernandez-Gallego, J.A.,Vergara-Díaz, O., Nieto-Taladriz, M.T., Araus, J.L. (2019) **UAV and ground image-based phenotyping: a proof of concept with durum wheat**. Remote Sensing 11:1244. DOIdoi.org/10.3390/rs11101244.

Peroni, L., Chartuni, E., Hummel, C.A., Neale, C.M., Gonçalves,I.Z., Filgueiras, R., Eugenio, F.C. (2020) **Potential of using spectral vegetation indices for corn green biomass estimation based on their relationship with the photosynthetic vegetation sub-pixel fraction**. Agricultural Water Management 240:106155.DOI doi.org/10.1016/j.agwat.2020.106155.

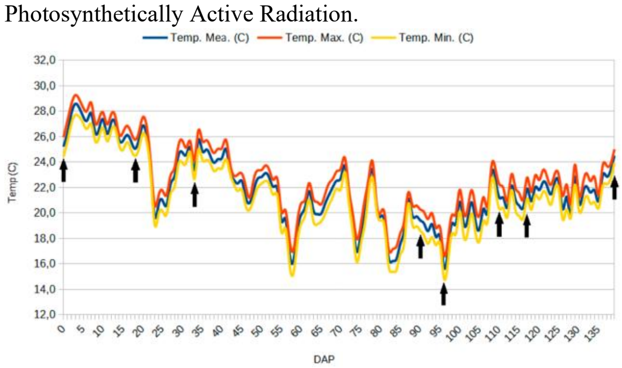
Ribeiro, H.J., Schwambach, D.A., Marcato, V.J., Cortez, J.W. (2021) **Vegetation indices and their correlation with second-crop corn grain yield in Mato Grosso do Sul, Brazil**. Agronomic Research 20:1195. DOI doi.org/10.18512/rbms2021v20e1195.

van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, M.C. (1997) Recomendações de Adubação e Calagem Para o Estado de São Paulo. Instituto Agronômico, Campinas.

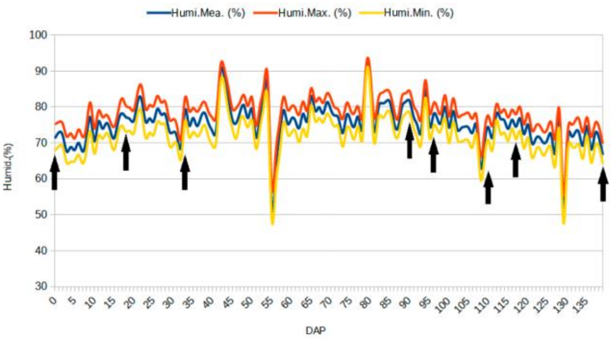
Zheng, R., Deng, M., Lv, D., Tong, B., Liu, Y., Luo, H. (2023) **Combined BSA-Seq and RNA-Seq reveal genes associated with the visual stay-green of maize (Zea mays L.)**. International Journal of Molecular Sciences 24:17617. DOI doi.org/10.3390/ijms242417617.

Supplementary Materials

Figure S1: Climatological values for each day after planting (DAP). (a) Temperature. (b) Humidity. (c) Total rainfall. (d) Photosynthetically Active Radiation.

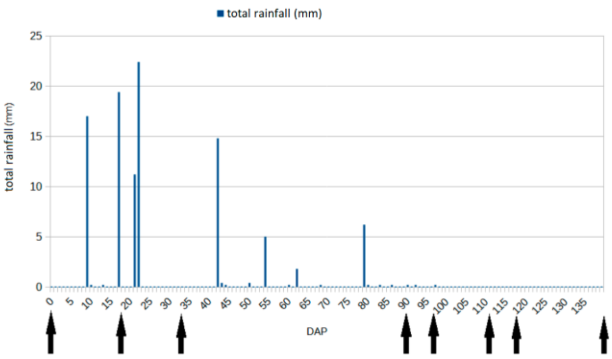


(a)



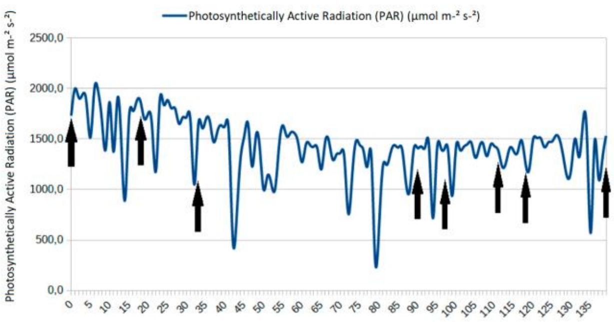
Humi.Mea. (%)
Humi.Max. (%)
Humi.Min. (%)
DAP

(b)



total rainfall (mm)
total rainfall (mm)
DAP

(c)



Photosynthetically Active Radiation (PAR) (µmol m⁻² s⁻²)
Photosynthetically Active Radiation (PAR) (µmol m⁻² s⁻²)

(d)