# RGB-derived vegetation indices as a tool to estimate soil coverage ability in durum wheat

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## Background

Crop varieties possessing traits such as early vigor, high tillering ability, plant height, and high anthesis biomass have a competitive advantage over weeds. Among cereals, durum wheat (*Triticum durum* Desf.) has the lowest competitive ability to face weeds, due to its weak early vigor that limits soil coverage during early-growth stage along with a reduced plant height and a low biomass at flowering. To overcame the above limitations, here we reported the ability of RGB-derived vegetation indices (VIs) from Unmanned Aerial Vehicle (UAV) for evaluating the soil coverage during the vegetative phase in a large collection of durum wheat varieties. To validate the RGB-derived indexes, two durum wheat varieties grown under different sowing rate and nitrogen application rate were chosen.

## Materials and Methods

Two field trials were carried out consecutively during four consecutive growing seasons (2016-2019) at the experimental farm of CREA-CI Research Centre for Cereal and Industrial Crops, Foggia, Italy (41°27'44.9"N 15°30'03.9"E). In the first field experiment (Exp.1) (2015-2016), 450 durum wheat varieties were grown in small plots (4 $m^{-2}$) with three replications in a randomized complete block design (RCBD). The second experiment (Exp. 2) was carried out over three growing-seasons (2016-17, 2017-18 and 2018-19) in 10 $m^{-2}$ plots. In detail, two durum wheat varieties (Natal and Nadif), previously selected as contrasting genotypes based on RGB vegetation indices values, were grown using two sowing rate (200 and 400 seeds $m^{-2}$) and six N doses (N0=0 kg $N^{-1}$; N1=60 kg $N^{-1}$; N2=90 kg $N^{-1}$; N3=120 kg $N^{-1}$; N4=180 kg $N^{-1}$; N5=240 kg $N^{-1}$). Aerial and ground phenotyping along with sampling were performed during the four growing seasons for both experimental field trials. In particular, UAV aerial digital RGB images, obtained with Zenmuse X5 settled on Matrice 100, were collected 4 times (FLY1-4) during the first experiment and 5 times on second (FLY1-5). RGB-based Green Area (GA), Greener Area (GGA), Normalized Green Red Difference Index (NGRDI), Triangular Greenness Index (TGI) and crop senescence index (CSI) were calculated during four consecutive growing seasons using Cereal Scanner, a tool belonging ImageJ software (REF). Statistical analysis and figures presented were performed on R studio ver. 2022.02. 0 (RStudio Team (2020), PBC, Boston, MA)

## Results

The PCA calculated using all the VIs recorded during the first year showed variability of the genetic materials under evaluation (Fig. 1). The first two axes of the PCA accounted for 95,78 % of the total variance. In detail, PC1 divided 2 genotypes, Nadif and Natal, clearly. The contrasting behavior between the two varieties was also evident, confirming our choice in selecting these two genotypes for the second validation experiment (Fig 2).

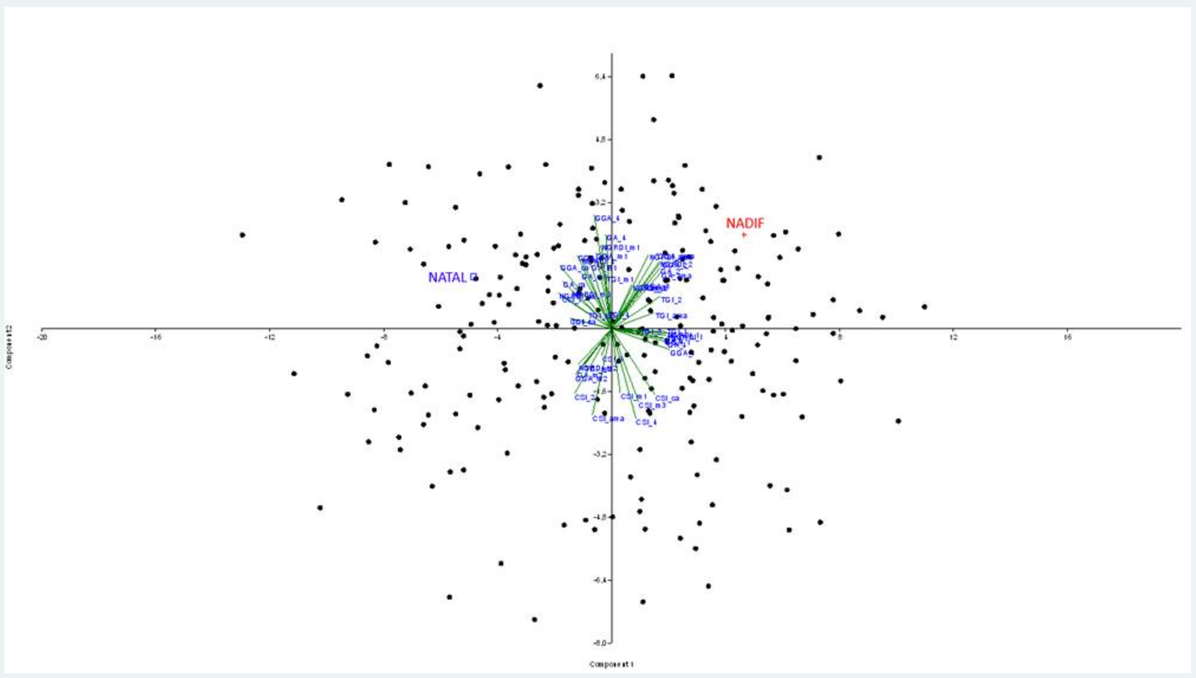


***Figure 1***. *PCA obtained using all the VIs recorded during the first field trial (2015-16 at Foggia, Italy). The black dots indicated the 450 durum wheat varieties; the green arrows indicated the vegetation indices. The two varieties, selected for the second field experiment, are shown in red (NADIF) and in blue (NATAL), respectively.*

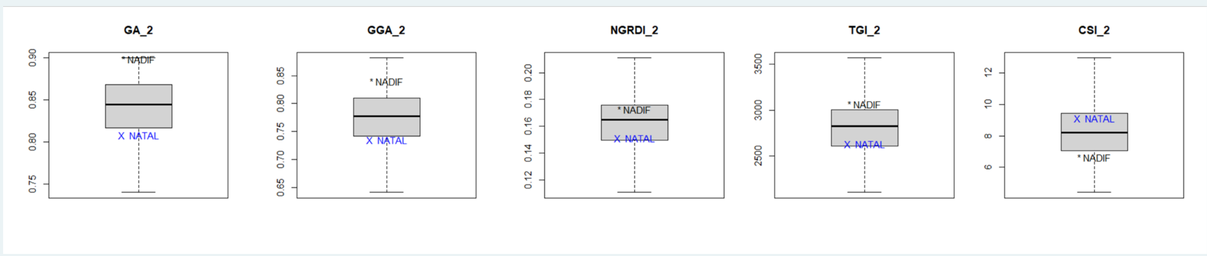


***Figure 2***. *Box plots derived from the images recorded with the second UAV flight (FLY2) during the tillering phase for all VIs. The two selected genotypes (NATAL and NADIF) are highlighted in each box plot.*

The ANOVA, conducted using the VIs values recorded during the second experiment, revealed levels of significance for all the factors (Tab. 1). All the VIs studied here showed a good discriminating capacity with respect to the observed varieties, fertilization and sowing densities, with the only exception for CSI index.

***Table 1***. *ANOVA Effect of variety, year, N doses and Sowing rate treatments on VIs evaluated in three-year-study.*

| VEGETATION INDICES (VIs) | VARIETY | BLOCK | YEAR | NITROGEN DOSES | SOWING RATE |
|---|---|---|---|---|---|
| GA | *** | n.s. | *** | ** | n.s. |
| GGA | *** | n.s. | *** | *** | n.s. |
| TGI | *** | n.s. | n.s. | ** | *** |
| NGRDI | *** | n.s. | ** | *** | ** |
| CSI | n.s. | n.s. | *** | n.s. | n.s. |

*n.s. = not statistically significant , *p<0.05, **p<0.01, ***p<0.001*

In the second experiment the multivariate analysis of variance (MANCOVA) confirmed the good discriminating capacity of the VIs for the two genotypes under investigation and for the seeding rate, starting from the tillering phase (η2=0.50), whereas for the N doses the differences were significant only from the heading stage, i.e., only after the absorption and utilization of N by the crop. For the two varieties, significance emerged after the third flight (FLY3), with a maximum of η2 coinciding with the FLY4 (Fig. 3). The heritability (h2) calculated for the five flights carried out over the three growing seasons followed the same trend as η2, with increasing values for the all VIs, with a decrease only in the FLY5 (Tab. 2).

***Table 2***. *Heritability values of the VIs calculated for all flights (FLY) conducted during the 2017-2019 growing seasons.*

| INDEX | FLY_1 | FLY_2 | FLY_3 | FLY_4 | FLY_5 |
|---|---|---|---|---|---|
| GA | 0.921 | 0.853 | 0.975 | 0.986 | 0.969 |
| GGA | 0.932 | 0.839 | 0.975 | 0.907 | 0.967 |
| TGI | 0.935 | 0.850 | 0.976 | 0.995 | 0.952 |
| NGRDI | 0.903 | 0.814 | 0.968 | 0.974 | 0.407 |
| CSI | 0.930 | 0.688 | 0.939 | 0.868 | 0.930 |

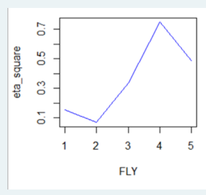


***Figure 3***. *η2 values (y-axis) calculated for the 5 flights carried out on the two varieties examined*

The PCA analysis and clustering performed on the VIs values recorded in FLY4 confirmed the best discriminating capacity between the two varieties NATAL and NADIF evaluated under different agronomic conditions (i.e., two sowing rates and five N levels) (Fig. 4).

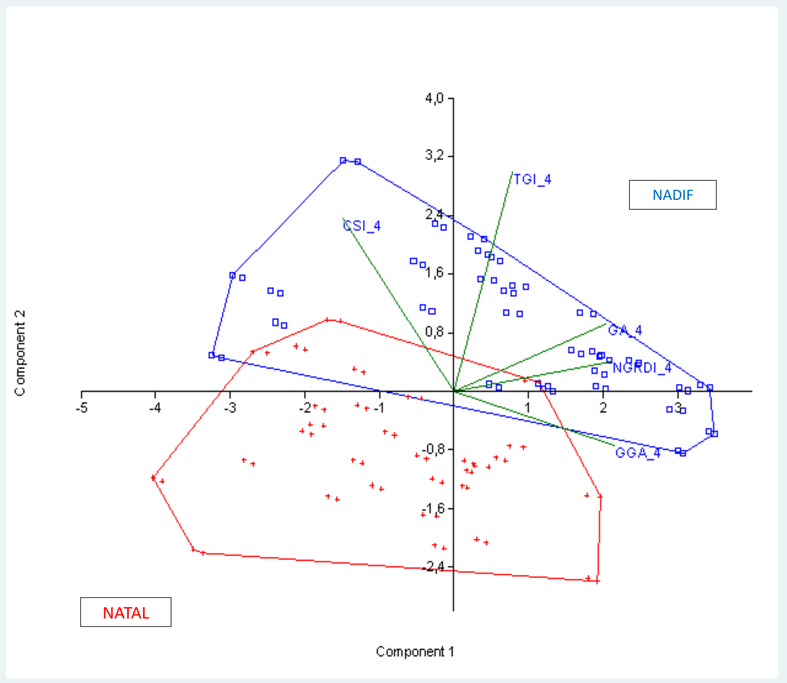


***Figure 4***. *PCA analysis and clustering of Vis registred for two durum wheat varieties grown under different agronomic conditions across three growing seasons.*

## Conclusion

Genetic correlations of RGB-derived indices vs. grain yield and other traits (plant height, heading date, flowering time, biomass at maturity) were also significant (data not shown). These results highlight the potential use of RGB-derived VIs for digital phenotyping to select durum wheat genotypes with higher soil coverage ability under different agronomic conditions.

## Acknowledgments

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