

## USING PRECISION AGRICULTURE AND REMOTE SENSING TECHNIQUES TO IMPROVE GENOTYPE SELECTION IN A BREEDING PROGRAM

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### ABSTRACT

Precision Agriculture (PA) and Remote Sensing (RS) technologies are increasingly being used as tools to assess crop and soil properties by breeders and physiologists. These technologies are showing potential to improve genotype selections over their traditional field measurements, by providing quick access to crop properties throughout the crop cycle and yield estimation. The objective of this work was to use vegetation indices (VIs) and soil apparent electrical conductivity ( $EC_a$ ) as predictor variables of yield. This information was obtained from a durum wheat yield trial, aiming to estimate yield of different genotypes under full and reduced irrigation. This work was carried out at CIMMYT's experiment station at Ciudad Obregón/Sonora, Mexico, during 2013 wheat crop cycle. There were four yield trials, two with reduced irrigation (RID) and two with full irrigation (FIG), which tested 112 different genotypes in a completely randomized design with three replications. A flight campaign took place, with six flights, once per week from March to April 2013, using a 6-channel multispectral camera with 10 nm FWHM filters onboard an airplane flying 300 m above ground yielding 0.3 m resolution. The  $EC_a$  data was collected just before sowing using an EM38 device in each plot. Twenty three different VIs ranging from chlorophyll, structural, red edge ratios and RGB indices were calculated using the multispectral images. A Pearson's correlation was done using the yield of the check genotypes of each experiment with the VIs of each image and  $EC_a$ , aiming to explore the potential of each variable on predicting yield. This approach was followed by a subset multiple regression method, using as predictive variables the VIs coefficients fitted to each genotype considering a quadratic effect plus  $EC_a$ , to fit the yield of each genotype in a training dataset, and then applied into a Bootstrap method in the cross validation dataset. The significant correlations among yield from the check genotypes and VIs from all images, plus  $EC_a$ , ranged

from -0.82 to 0.73 in the RID and from -0.70 to 0.60 in the FIG experiment. The correlation coefficients between measured *versus* predicted yield by the models got mean values of 0.51 (RID) and 0.68 (FIG) using the cross validation dataset, being 0.27 (RID) and 0.47 (FIG) of r-squared, indicating that the use of different VIs together may improve the yield prediction of breeding experiments.

**Keywords:** wheat, multispectral images, soil apparent electrical conductivity, multiple regressions, bootstrapping.

## INTRODUCTION

The agricultural research sector has been potentially important, aiming to develop new technologies and management knowledge to sustainably increase the food productivity, to ensure global food security and decrease poverty. Wheat is one of the most important crops into this scenario, being among the two most important cereal commodities produced worldwide. Inside this context, Mexico is one of the most important producers of wheat, harvesting more than 500,000 hectares in 2012, totaling 3.2 million of tonnes (FAO 2013). One important pillar of this platform is a wheat breeding program, which should provide varieties able to adapt to different environments.

The Global Wheat Program (GWP) at the International Wheat and Maize Improvement Center (CIMMYT) has been working on the development of wheat germplasm for more than 50 years, and recent estimates indicate that CIMMYT derived genotypes are planted on more than 64 million hectares in developing countries, representing more than 75% of the area planted to modern wheat varieties in those countries (CIMMYT 2014).

Precision Agriculture (PA) and Remote Sensing (RS) technologies can play an important role, in the identification, with high accuracy and density, of soil and plant properties inside breeding plots, and thus allowing for a quicker and less labor intensive selection of genotypes.

Proximal soil sensing has become an increasingly common and essential element of PA (Bramley 2009, Bramley and Trengove 2012). Most commonly, this has involved measurements of apparent electrical conductivity ( $EC_a$ ; Corwin and Plant 2005 and references therein) using electromagnetic induction (EMI; e.g. Hedley et al. 2004), which gives information related to soil physical properties, broadly used in PA to delineate management zones of yield potential (Bramley 2009).

In parallel, RS technologies have become in the recent years more inexpensive which has improved the availability and flexibility of acquiring images. As a consequence, vegetation indices (VI) such as the Normalized Difference Vegetation Index (NDVI), has been broadly used to correlate with crop yield in agricultural experiments (Mullan 2013 and references therein). Whereas we assume that the results presented by those authors were considered useful, it is well documented that NDVI data saturate at high Leaf Area Index (LAI) values, and is also affected by other factors such as soil background, canopy shadows,

illumination, atmospheric conditions and variation on leaf chlorophyll concentration (Zarco-Tejada et al. 2005). Moreover, there is not much use of predictive models and their validation besides the use of simple correlations. So here we present how VIs and soil  $EC_a$  may be able to predict grain yield in breeding experiments. This approach is important in terms of improving the efficiency of a breeding program, which may be done through a rapid assessment of crop characteristics and yield, decreasing field labor and time requirements.

The objective of this work was to use VIs and soil  $EC_a$  as predictive variables of yield, from a yield trial with different genotypes under full and reduced irrigation.

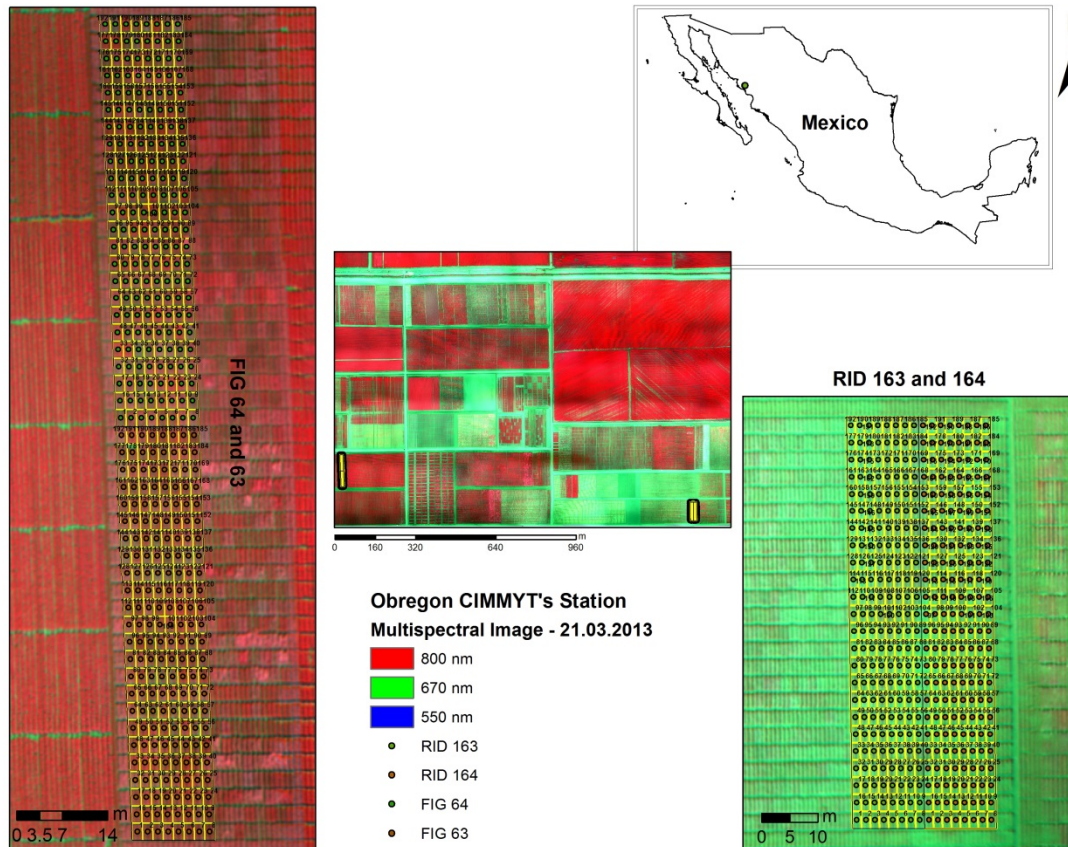
## **MATERIAL AND METHODS**

### **Field site and data collection**

The experiments were carried out in the CIMMYT's experiment station at Ciudad Obregón/Sonora, Mexico, during 2013 wheat crop cycle (27.383848° N and 109.923878° W). It consisted of four yield trials, two with reduced irrigation (RID) and two with full irrigation (FIG), which tested 112 different genotypes plus check genotypes in a completely randomized design with three replications (Fig. 1). Thus, each experiment (RID) totaled 192 observations, split into three replications, which each one contained 56 genotypes plus eight checks; the same design was used in the FIG trial. The experiments were sowed on November/December 2012 and harvested on May 2013. Yield was measured in each plot according the protocol described by Pask et al. (2012).

The soil sensing of apparent electrical conductivity ( $EC_a$ ) was done through electromagnetic induction (EMI) just before sowing using an EM38 device on both dipoles, taking one measurement in the center of each plot.

A flight campaign took place, with six flights, once per week from March to April 2013, using a 6-channel multispectral camera with 10 nm FWHM filters onboard an airplane flying 300 m above ground.



**Fig. 1. CIMMYT'S experiment station at Ciudad Obregón; Full Irrigated (FIG) and Reduced Irrigated (RID) trial.**

The image resolution is  $1280 \times 1024$  pixels with 10 bit radiometric resolution and optics focal length of 8.5 mm, yielding an angular field of view (FOV) of  $42.8^\circ \times 34.7^\circ$  and 0.3 m pixel spatial resolution at 300 m flight altitude. Atmospheric correction and radiometric calibration methods were applied to the imagery to calculate the spectral reflectance. Radiometric calibration was conducted in the laboratory using coefficients derived from measurements made with a uniform calibration body (integrating sphere, CSTM-USS-2000C Uniform Source System, LabSphere, NH, USA) at four levels of illumination and eleven integration times. Radiance values were converted to reflectance using the total incoming irradiance simulated with the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS) (Gueymard, 1995, 2005) using aerosol optical depth at 550 nm measured with Micro-Tops II sunphotometer (Solar LIGHT Co., Philadelphia, PA, USA). This radiative transfer model was previously used in other studies such as Berni et al. (2009) and Suárez et al. (2010). The geometric calibration was conducted using Bouguet's calibration method (Bouguet 2001) in order to calculate the intrinsic camera parameters (Berni et al. 2009).

## Data analysis

Twenty three different VIs ranging from chlorophyll, structural, red edge ratios and RGB indices (Table 1) were calculated using all the wavelengths (550, 670, 700, 710, 750, 800 nm) from each multispectral images. In parallel, the yield adjustment of each genotype was done by means of Restricted Maximum Likelihood (REML), these adjusted means were used as response variables into the modelling process further on.

As a first step, a Pearson's correlation was done using the yield of the checks genotypes ( $n=48$ ) of each experiment with the VIs of each image, thermal data and  $EC_a$  ( $p \leq 0.05$ ). Line plots were done for each VIs across the images by experiment, aiming to check the genotypes behavior across time.

As there were a total of 144 predictive variables (23 VIs + Thermal x 6 images) using the images; each genotypes yield were fitted by VIs across time through regression analysis considering a quadratic effect (yield  $\sim$  intercept + VIs + VIs<sup>2</sup>), thus selecting the fits coefficients (intercept, linear and quadratic effect) of each VI. This process was done prior to the modelling process aiming to reduce the total number of predicted variables.

Each coefficient (plus  $EC_a$  at both depths) was then used as predictive variables into a subset multiple regression approach by means of partial least square method, using the adjusted yields of each genotype as response variable. All these data process were done for each type of experiments separately (RID and FIG). The dataset of each experiment was randomly split into 70% for model fitting (training dataset; TD) and 30% for cross validation (CV). The fitted model was then applied in the 30% of the dataset into Bootstrap method (Efron 1979) with 10 thousand interactions, obtaining the distribution of the correlation between measured and predicted yield and r-squared ( $R^2$ ) of it.

Table 1. Vegetation Indices calculated from multispectral imagery.

Vegetation Index	Equation	Reference
<b>Structural Indices</b>		
Normalized Difference Vegetation Index (NDVI)	$\frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red}}$	Rouse et al. (1974)
Renormalized DVI (RDVI)	$\frac{R_{800} - R_{670}}{\sqrt{R_{800} + R_{670}}}$	Rougean and Breon (1995)
Optimized Soil-Adjusted Vegetation Index (OSAVI)	$\frac{(1 + 0.16) * (R_{800} - R_{670})}{(R_{800} + R_{670} + 0.16)}$	Rondeaux et al. (1996)
Simple Ratio Index (SR)	$\left(\frac{R_{NIR}}{R_{red}}\right)$	Jordan (1969); Rouse et al. (1974)
Modified Simple Ratio (MSR)	$\frac{R_{NIR}}{R_{red}} - 1 / \left(\frac{R_{NIR}}{R_{red}}\right)^{0.5} + 1$	Chen (1996)
Modified Triangular Vegetation Index (MTVI <sub>1</sub> )	$1.2 * [1.2 * (R_{800} - R_{550}) - 2.5 * (R_{670} - R_{550})]$	Haboudane et al. (2004)
Modified Triangular Vegetation Index (MTVI <sub>2</sub> )	$\frac{1.2 * [1.2 * (R_{800} - R_{550}) - 2.5 * (R_{670} - R_{550})]}{\sqrt{(2 * R_{800} + 1)^2 - (6 * R_{800} - 5 * \sqrt{R_{670}}) - 0.5}}$	Haboudane et al. (2004)
Modified Chlorophyll Absorption in Reflectance Index (MCARI <sub>1</sub> )	$1.2 * [2.5 * (R_{800} - R_{670}) - 1.3 * (R_{800} - R_{550})]$	Haboudane et al. (2004)
Modified Chlorophyll Absorption in Reflectance Index (MCARI <sub>2</sub> )*	$\frac{1.2 * [2.5 * (R_{800} - R_{670}) - 1.3 * (R_{800} - R_{550})]}{\sqrt{(2 * R_{800} + 1)^2 - (6 * R_{800} - 5 * \sqrt{R_{680}}) - 0.5}}$	Haboudane et al. (2004)
<b>Chlorophyll Indices</b>		
Triangular Vegetation Index (TVI)	$0.5 * [120 * (R_{750} - R_{550}) - 200 * (R_{670} - R_{550})]$	Broge and Leblanc (2000)
Modified Chlorophyll Absorption in Reflectance Index (MCARI)	$[(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * \frac{R_{700}}{R_{670}}$	Daughtry et al. (2000)
Transformed CARI (TCARI)	$3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * \frac{R_{700}}{R_{670}}$	Haboudane et al. (2002)
TCARI/OSAVI	$\frac{3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * \frac{R_{700}}{R_{670}}}{(1 + 0.16) * (R_{800} - R_{670}) / (R_{800} + R_{670} + 0.16)}$	Daughtry et al. (2000); Rondeaux et al. (1996)
MCARI/OSAVI	$\frac{[(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * \frac{R_{700}}{R_{670}}}{(1 + 0.16) * (R_{800} - R_{670}) / (R_{800} + R_{670} + 0.16)}$	Daughtry et al. (2000); Rondeaux et al. (1996)
Gitelson and Merzlyak (GM1)	$\left(\frac{R_{750}}{R_{550}}\right)$	Gitelson and Merzlyak (1997)
Pigment specific simple ratio for chlorophyll a (PSSRa)*	$\left(\frac{R_{800}}{R_{680}}\right)$	Blackburn (1998)
<b>Red Edge ratios</b>		
Zarco-Tejada and Miller	$\left(\frac{R_{750}}{R_{710}}\right); \left(\frac{R_{750}}{R_{700}}\right); \left(\frac{R_{750}}{R_{670}}\right); \left(\frac{R_{710}}{R_{700}}\right); \left(\frac{R_{710}}{R_{670}}\right)$	Zarco-Tejada et al. (2001)
<b>RGB Indices</b>		
Red; Green	$\left(\frac{R_{700}}{R_{670}}\right); \left(\frac{R_{550}}{R_{670}}\right)$	Zarco-Tejada et al. (2005)

\* used 670nm instead of 680nm.

## RESULTS AND DISCUSSION

The correlation analysis among all VIs, temperature and EC<sub>a</sub> with yield of the check genotypes ranged from -0.82 to 0.73 in the RID and -0.70 to 0.60 in the FIG experiment. Table 2 shows the best 10 correlations coefficients for both experiments. On RID, temperature had the best correlations with yield, while on FIG, TCARI and MCARI indices were the best correlated.

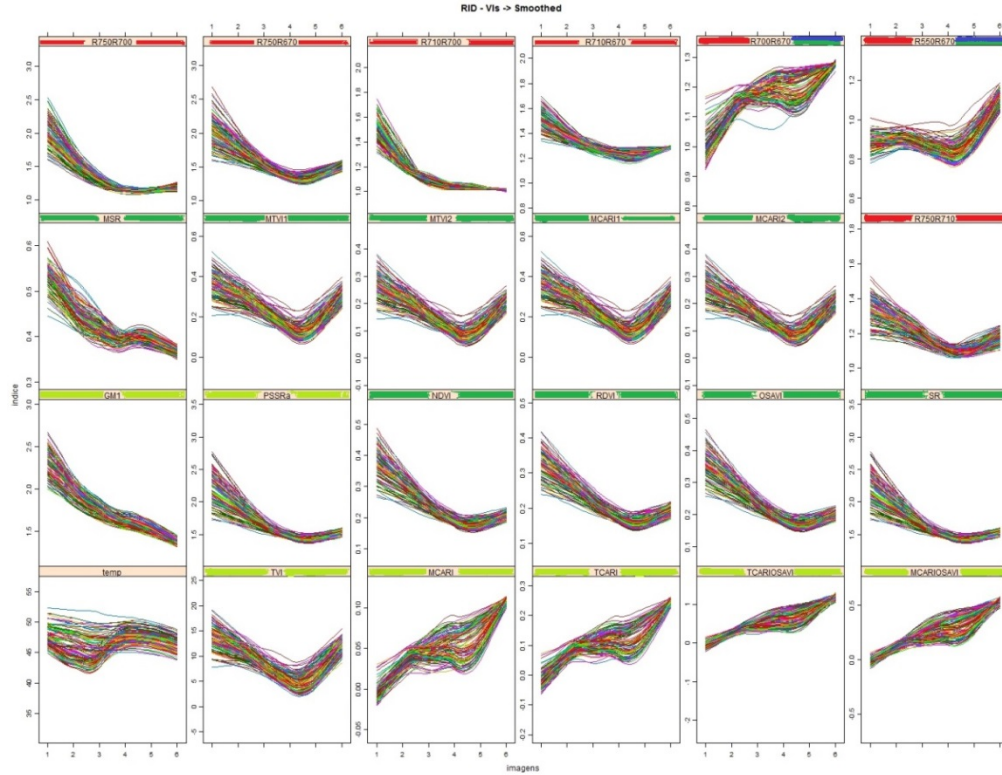
Temperature showed to be highly negatively correlated with yield in reduced irrigation environment (RID), followed by NDVI as highly positively correlated. On FIG the correlation between temperature and yield varied between -0.50 to 0.06 across images acquired during the crop cycle, being the higher coefficient from the image acquired on 24 April 2013, same image as on the RID experiment. Cossani et al. (2012) got similar results analysing canopy temperature (measured by infrared thermometry gun; IR) with grain yield, getting -0.66 coefficient of correlation under drought environment, and -0.58 under irrigated environment condition. Even though the authors claim the speed of measurements, simplicity and low cost, as advantages of the use of the IR gun, this equipment may compromise the reliability of the data when used in extensive field plots which need hours of measurements – this is not a problem when thermal sensors are used in remote sensing platforms. TCARI and MCARI had the highest correlation coefficients (-0.70; -0.69) with yield in the FIG experiment, although NDVI reached slightly smaller coefficient (-0.62). Although throughout the literature it is possible to check many other studies reporting high correlations between NDVI and grain yield (Ball and Konzak 1993; Inman et al. 2007; Marti et al. 2007; Raun et al. 2001; Reynolds et al. 2001; Royo et al. 2003; Prasad et al. 2007), there is still a lack of information on the potential use of VIs together into models for predicting yield. Those correlation coefficients just indicated the relationship between the yield from the check genotypes and VIs, it doesn't necessarily mean power of prediction to different genotypes.

Table 2. Best 10 correlation coefficients between VIs and yield checks

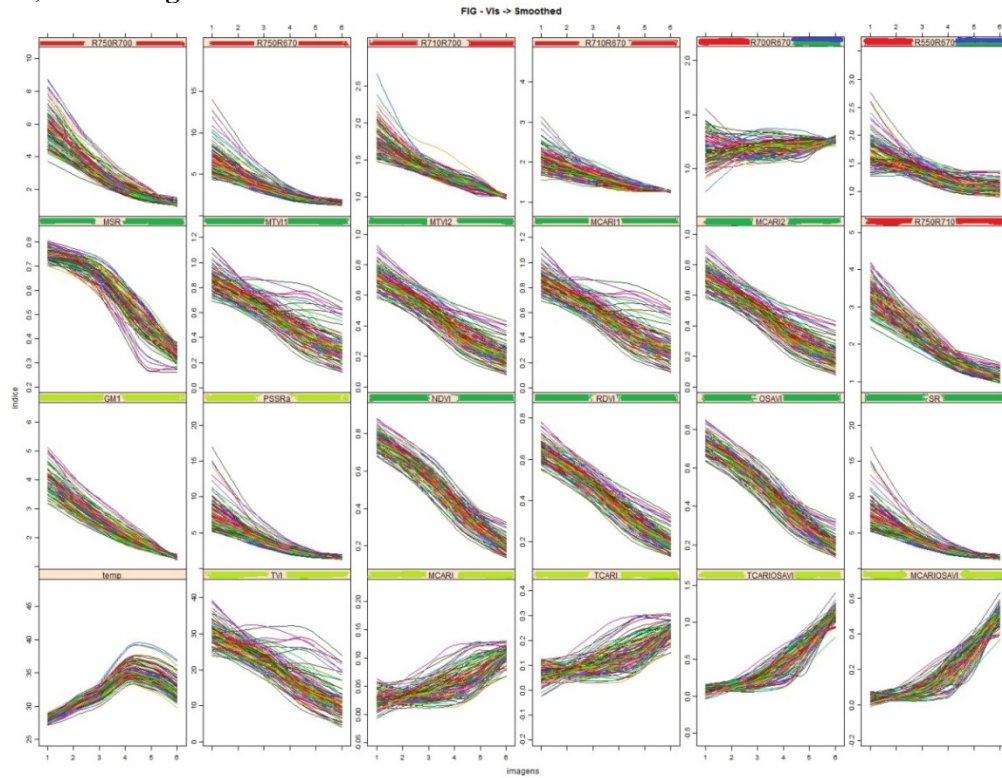
<b>Checks - RID</b>	<b>Yield</b>	<b>Checks – FIG</b>	<b>Yield</b>
Temp - 130424	-0.82*	TCARI – 130411	-0.70*
Temp - 130403	-0.80*	MCARI – 130411	-0.69*
Temp - 130411	-0.78*	R550/R670 – 130411	-0.68*
Temp - 130314	-0.77*	MTVI <sub>2</sub> - 130411	-0.66*
Temp - 130321	-0.77*	MCARI <sub>2</sub> - 130411	-0.66*
Temp - 130326	-0.75*	MTVI <sub>1</sub> - 130411	-0.65*
NDVI - 130326	0.73*	MCARI <sub>1</sub> - 130411	-0.65*
PSSRa - 130326	0.73*	TVI - 130411	-0.63*
SR - 130326	0.73*	NDVI - 130411	-0.62*
OSAVI - 130326	0.73*	OSAVI - 130411	-0.62*

\* - statistically significant at 0.01 probability; Temp – temperature extracted from the thermal images; dates are expressed as YYMMDD; R550/R670 – Green VI.

On Figures 2 and 3 is possible to see each genotype response (y axis) across time/images (x axis) for each VIs on both experiments.



**Fig. 2. Line plots of VIs per genotype (RID). Light green label – Chlorophyll Indices; Dark green label – Plant structure indices; Red label – Red Edge ratios; Red, blue and green label – RGB ratios.**



**Fig. 3. Line plots of VIs per genotype (FIG). Lighter green label – Chlorophyll Indices; Darker green label – Plant structure indices; Red label – Red Edge ratios; Red, blue and green label – RGB ratios.**



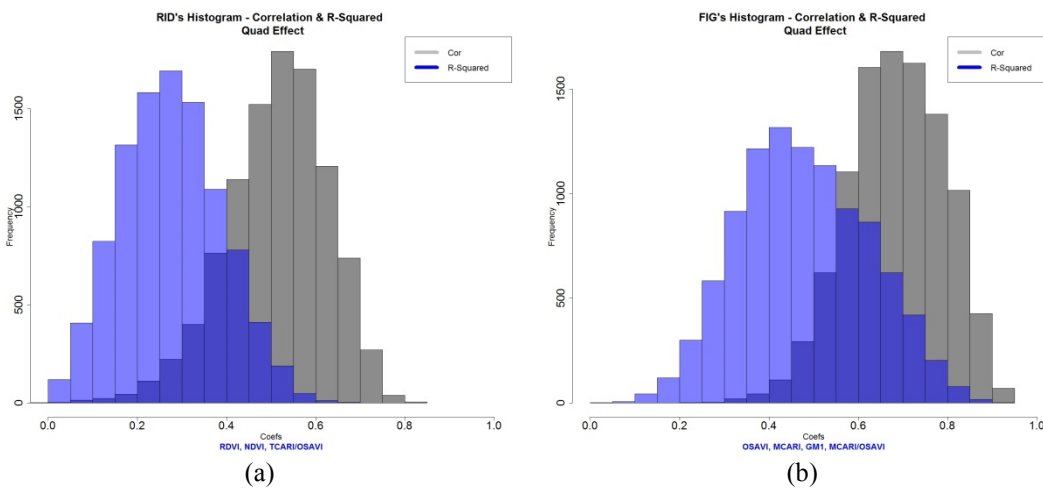
Through Figures 2 and 3 it is possible to visualize the different behaviours of the genotypes across the images, also showing the temporal trend of it, supporting the idea of fitting each genotypes yield by VIs across time considering a quadratic effect and grabbing the fitted coefficients, reducing the number of predictive VI variables to 72 (against the originally 144) without losing temporal information of it.

The REML analysis for adjusting the mean yield across the experiment showed significant  $p$ -value on  $f$ -test, considering just the experimental design on RID and the interaction of the plots coordinates as random effect on FIG.

On the modelling approach, the adjusted yield means were used as response variable against VIs coefficients and  $EC_a$  by means of Subset Regression. It was specified a maximum of four variables to get into the model, as we chose to include the full VI information (intercept, linear and quadratic effect coefficient) of those which just one or two coefficients were selected. This was done aiming to keep a ratio of 3:1 between CV observations and predict variables.

The RID yield model using TD got 0.46 of adjusted r-squared ( $R_{adj}$ ), with all parameters statistically significant at 0.01 of probability. The VIs selected were RDVI, NDVI and the ratio TCARI/OSAVI. The FIG yield model got 0.56 of  $R_{adj}$ , also with all model's parameters statistically significant at 0.01 of probability. The VIs selected were OSAVI, MCARI, GM1 and the ratio MCARI/OSAVI. If used just NDVI to fit a yield model using the TD of the RID experiment, which was the experiment where NDVI was added in the model together with other VIs, the  $R_{adj}$  decrease from 0.46 (prior cited) to 0.09, strongly suggesting that the use of more VIs together may improve yield prediction.

As we were interested to check the predictive power of those models, they were applied in the CV dataset, into a Bootstrapping method with 10 thousand interactions. On Figure 4 ('a' and 'b') is possible to visualize the distribution of the correlation coefficients between measured and predicted yield, obtained as output of the Bootstrapping interactions for both experiment's model.



**Fig. 4. Histograms of correlation coefficients and  $R^2$  from RID (a) and FIG (b) experiments.**

The distribution of predictions' coefficients (measured *versus* predicted yield) on RID considering 95% confidence interval (CI) is 0.26-0.70, with mean of 0.51; and 0.07-0.50 to  $R^2$  with mean as 0.27. FIG had CI between 0.46-0.87 and 0.21-0.75 with means of 0.68 and 0.47 to correlation coefficients and  $R^2$ , respectively. Zarco-Tejada et al. (2005) tested different hyperspectral indices related to vegetation structure and canopy chlorophyll concentration, aiming to within-field yield variability in cotton crop, indicating that those vegetation indices may be used as complementary information to NDVI, for describing yield variation. Those authors indicated that VIs such as RDVI, TCARI, OSAVI, and MCARI were good indicators to canopy variability related to cotton yield. In the present study, with exception of GM1, those same VIs (single and/or as ratios) were selected through the modelling approach as predictable variables to estimate yield of different genotypes of wheat, indicating its potential on estimating yield on breeding experiments. Further analysis should be done using multi sites-cycles data, to check if the best VIs are consistent over years, if more VIs could be added into the modelling and which would be the best crop stages to acquire RS images.

## CONCLUSION

Although the models got from low to high correlations and  $R^2$  on the cross-validation throughout several interactions, this study indicates that grouping VIs related to plant structure and chlorophyll content, such as RDVI, TCARI, OSAVI, MCARI and GM1, into models and using its temporal information across crop cycle, it may be complementary information to traditionally VIs such as NDVI, as indicated by Zarco-Tejada et al. (2005), improving the power of prediction of yield in breeding experiments and as a consequence being able to select genotypes using such technologies.

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