



## **INnovations in plant Varlety Testing in Europe**

### **Deliverable D1.4**

## **D1.4: Bioindicators for wheat tolerance to water stress (M40)**

## Technical References

Project Acronym	INVITE
Project Title	INnovations in plant Variety Testing in Europe
Project Coordinator	François Laurens
Project Duration	60 months
Deliverable No.	D1.2
Dissemination level <sup>1</sup>	
Work Package	WP 1 - Crop characteristics and bioindicators associated with plant sustainability and DUS criteria
Task	T 1.1 - Identification of novel and more comprehensive indicators for crop resilience and adaptation to abiotic stress
Lead beneficiary	Institut de la Recerca I Tecnologies Agroalimentaries (IRTA)
Contributing beneficiary(ies)	7-IRTA, 22-ACTA
Due date of deliverable	31 October 2022
Actual submission date	07 December 2022

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PP = Restricted to other programme participants (including the Commission Services)

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CO = Confidential, only for members of the consortium (including the Commission Services)

## Document history

V	Date	Beneficiary	Author
1	09/11/22	IRTA	Marta Da Silva
2	15/11/2022	INRAE	François Laurens
3	28/11/2022	IRTA	Marta Da Silva
4	30/11/2022	INRAE	François Laurens



## Summary

Field trials were conducted in Spain and France in 2020-2021 under irrigated and rainfed conditions. The major bioindicators of drought tolerance under Mediterranean conditions were NDVI (Normalized Difference Vegetation Index) and Canopy temperature at around anthesis. The most drought tolerant varieties were Complice, Winner and Rubisco with lower Canopy temperatures at anthesis, higher NDVI and transpiration values, early to medium heading dates.



## Table of content

<b><u>1 INTRODUCTION .....</u></b>	<b><u>5</u></b>
<b><u>2 MATERIALS AND METHODS .....</u></b>	<b><u>6</u></b>
<b><u>3 RESULTS.....</u></b>	<b><u>13</u></b>
<b><u>4 CONCLUSION.....</u></b>	<b><u>17</u></b>



# 1 Introduction

Key adaptation traits associated with yield have been widely reported by adjusting wheat maturity to match the growing season. Late-maturing varieties are not suitable for semi-dry to dry regions and the introduction of early maturing varieties has been successfully at least partially to increase yields in Spain and southern France.

Water availability is the most important factor affecting yield also known as yield potential. Breeding programs targeting adaptation to water stress have selected lines that develop and mature over the growing season to maximize yield potential. Frequently, biomass and yield correlate positively (but not always), and breeders aim at matching the vegetative stages of crop development with the months when rainfall is more abundant. Exceptions to this, when winter temperatures are very low with high risk of cold damage, the crop cycle, particularly around heading time must be delayed to escape this early Spring low temperatures. As such, breeders must find an equilibrium for the growth cycle of new varieties that can escape cold damage but can withstand terminal drought and heat. Another exception is when water for irrigation is abundant and can be applied to the crop during terminal drought and heat periods; otherwise, early maturing lines are needed. In temperate regions with winter rainfall but hot dry summers, such as in Mediterranean-type climates, autumn sowing and winter growth can be used to build biomass but the early maturity, to allow harvesting before the summer drought, is important. Breeders have been tuning the crop development to optimize the viable growth period for the normal growing season in their region.

While the crop cycle strategy to fit climate patterns has been crucial to increase yields, the erratic nature of climate with changes in precipitation distribution and abnormal temperatures really complicates our capacity to keep yields up and novel strategies must be developed particularly, by exploring options to increase the efficiency of water use and minimize the impact of factors that might reduce the yield stability.

The aim of Task 1 was to identify novel and more comprehensive indicators for crop resilience and adaptation to abiotic stress (M1-M40). More specifically, Task 1.1 searched for wheat root and aboveground traits relevant for crop adaptation to water scarcity in France and Spain. The same lines were evaluated in both countries (IRTA for Spain and ARVALIS for France) and a range a measurements has been done to assess the behaviour of these lines against water stress: roots architecture traits, airborne multispectral and thermal imagery, physiological traits related to plant growth (early vigor, biomass production and allocation, yield and yield components) and photochemical properties (chlorophyll content, spectral indices).



## 2 Materials and Methods

### 2.1 Selection of varieties using yield stability indexes

see D1.2 for the selection of genotypes



## 2.2 Field trials

We evaluated these selected lines as explained in D1.2 in field in order to better understand the response of bread wheat varieties to drought stress. The network was composed by 2 field trials (Figure 4) in the Arvalis station of Gréoux-Les-Bains (South-East France) and IRTA station of Sucs (North-East Spain).

We evaluated 22 varieties (VAR, list above) in the field under well irrigated (IRRIGATED) and rainfed conditions (water deficit conditions = WAT\_STR) with 3 replicates of 2 m wide x 12 m long plots in Gréoux les Bains (04-France) and with 2 replicates of 1.2 m x 8 m plots in Sucs (Spain)

- *Alpha lat design*
- *Irrigation meters (2 per site)*
- *Seed treated with (PROTIOCONAZOL 25% + TEBUCONAZOL 15%)*
- *Target seed Rates: 450 seeds/m<sup>2</sup> (6 plots 1.750g of each variety sent to France).*

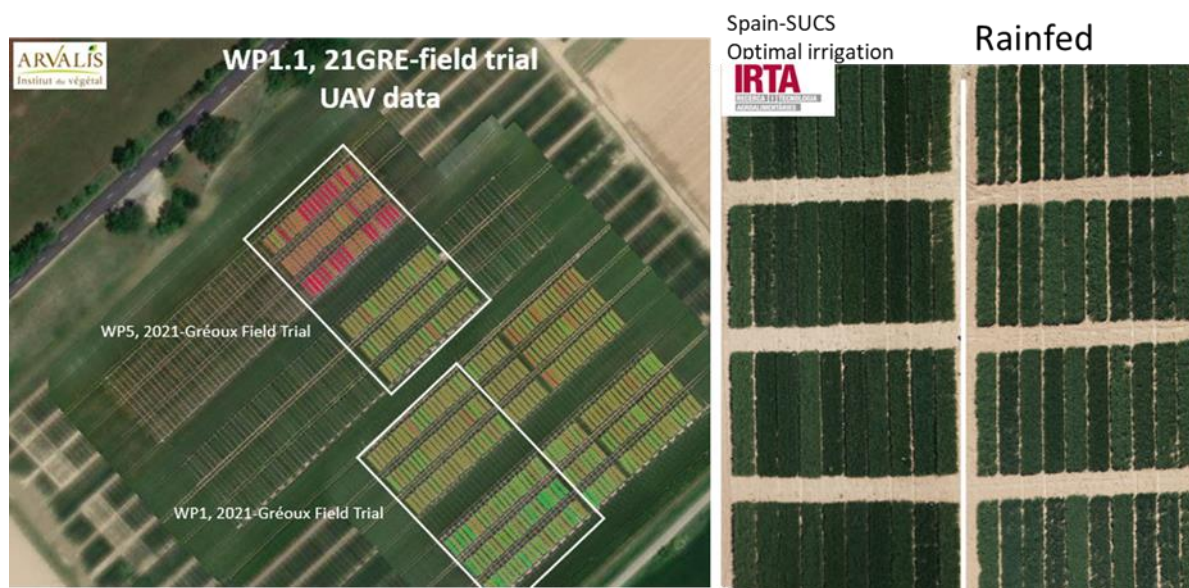


Figure 4 : Aerial picture of Gréoux les Bains platform in 2021 on the left and IRTA trials in Sucs- Lleida in Spain 2021 on the right.

## 2.3 Measurements

### 2.3.1 Agronomical traits

The varieties were evaluated for their early vigor, heading date, plant height, light interception, Leaf Area Index (LAI), yield and yield components (ear density, specific weight, protein content, Thousand Kernel Weight).

### 2.3.2 Root traits

In Gréoux field trial, for each wheat variety in the field trials, we measured the root length density per depth at 9 dates, imaging roots with a scanner into a transparent tube (named Minirhizotron, see Figure 5), plus *root grid density and diameter per depth*, in the irrigated and water stressed plots.



Figure 5: Minirhizotron tubes on Gréoux les Bains field trial

In Sucs, Spain only two varieties (Nogal and Marcopolo) were tested for root biomass at three depths (0-30, 30-60 and 60-90) in water stressed plots using a Giddings hydraulic soil corer coupled to a tractor, cutting the cores and washing roots from the sampled cores.



### 2.3.3 Sensor indexes

Biophysical parameters of the above ground vegetation (Green Fraction, Leaf Area Index, Fraction of Absorbed Photosynthetically Active Radiation -FAPAR, plant height, spectral indices: Chl, among other) have been estimated with multispectral and RGB high-resolution images.

In Gréoux-Les-Bains, we used DJI phantom4 (multispectral UAV), DJI Mavic pro (RGB UAV) and the Phénomobile (which carrying RGB camera, LIDAR and spectroradiometers) 8 times during the whole campaign.

In Sucs, we used FLIR SC655 (Thermal), Tetracam MACAW (Multispectral UAV) and Sony Alpha A6000 (RGB UAV) cameras 3 times during grain filling. Aerial images were used to estimate crop evapotranspiration and crop water stress index of different varieties using surface energy modelling approaches. Additional measurements of plant height, radiation interception and LAI were conducted in check plot for calibration.

### 2.3.4 Environmental measurements

In Greoux : we used tensiometers probes to manage irrigation and a fine mapping of the soil water holding capacity.

In Sucs : we selected high yielding and low yielding varieties check for soil water measurements with Neutron probe (apply PVC tubes before sowing in 4 plots) and measures of soil water content at sowing, at flights and at harvest.

In both site, local weather was assessed with classical measurements (temperature, rain...).



## 3 Results

### 3.1 Identification of potential bioindicators of drought tolerance in wheat

The hypothesis tested herein includes yield under drought as the main value trait for drought tolerance. Other parameters were measured that provide information about adaptation of the crop at different stages of development particularly at anthesis, and post-anthesis or the grain filling period that together culminate and contribute to final grain yield. Several parameters measuring chlorophyll content, total green canopy biomass (evaluated with its proxy NDVI - Normalized Difference Vegetation Index), canopy temperature as relevant proxy of root water extraction capacity and agronomic traits have shown significant correlation with yield as shown in Table 3 and Figures 6 and 7.

**Table 3. Correlation Matrix of potential drought tolerance bioindicators in 22 wheat varieties from Spain and France, grown under irrigated and rainfed conditions (average of trials conducted in Spain and France). Pearson correlation coefficients are shown and associated probabilities of correlation. NDVI (Normalized Difference Vegetation Index), TIR (Canopy Temperature in Celsius), TKW (Thousand Kernel Weight)**

Correlation Matrix	Irrigated		Rainfed	
	Yield (Ton/Ha)		Yield (Ton/Ha)	
Bioindicator Drought Tol	Pearson Coefficient	probability	Pearson Coefficient	probability
April07_NDVI	-0.0706	0.7548	<b>0.6089</b>	<b>0.0026</b>
April07_TIR_(oC)	-0.1364	0.5449	<b>-0.4999</b>	<b>0.0178</b>
April19_NDVI	-0.1432	0.5249	<b>0.5844</b>	<b>0.0043</b>
April19_TIR_(oC)	0.1982	0.3765	<b>-0.4926</b>	<b>0.0198</b>
May28_NDVI	-0.2303	0.3025	<b>0.5287</b>	<b>0.0114</b>
May28_TIR_(oC)	-0.265	0.2334	-0.0251	0.9117
Heading Date (Days)	<b>-0.4323</b>	<b>0.0445</b>	0.2976	0.1785
Maturity Date (days)	-0.3201	0.1464	0.3819	0.0795
Height (cm)	-0.2731	0.2189	0.205	0.3601
Lodging (%)	<b>-0.4557</b>	<b>0.0331</b>	0.3024	0.1714
Biomass (g)	0.1266	0.5744	0.2036	0.3634
Number of Grains m-2	0.1525	0.498	0.3928	0.0705
Number of stems m-2	<b>-0.4542</b>	<b>0.0337</b>	0.0708	0.7542
Grain Filling Duration (Days)	<b>0.4137</b>	<b>0.0556</b>	-0.0792	0.7262
TKW (g)	<b>0.4997</b>	<b>0.0179</b>	0.2493	0.2633
Protein (%)	<b>-0.7008</b>	<b>0.0003</b>	<b>-0.5012</b>	<b>0.0175</b>



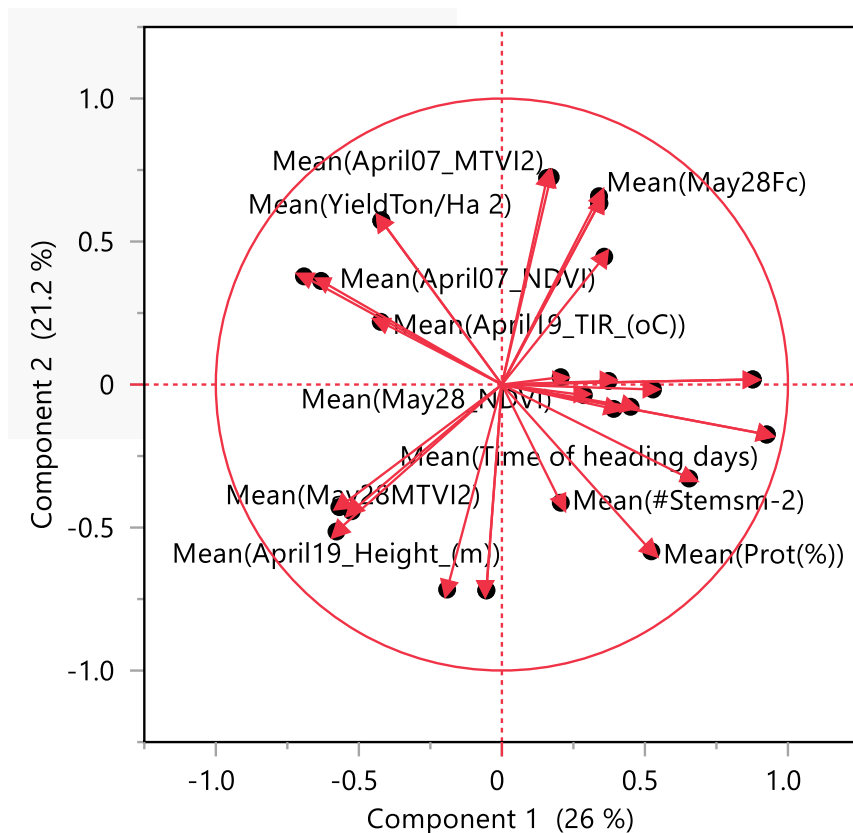


Figure 6. Principal component analysis of yield bioindicators under irrigated conditions of 22 wheat varieties (widely grown in Spain and France).

Under irrigated conditions the traits that best correlated with yield were mainly the agronomic traits defined at heading and grain filling stages (the reproductive stage) and include heading date (negative), Lodging (negative impact on grain yield), number of stems m<sup>-2</sup> (negative), grain filling duration (positive), thousand kernel weight (positive) and Protein content (negative). None of the vegetation parameters (at around heading and anthesis) showed effects on grain yield. These results indicated that water requirements were fulfilled, and yield did not depend greatly on the difference between varieties for vegetative traits.



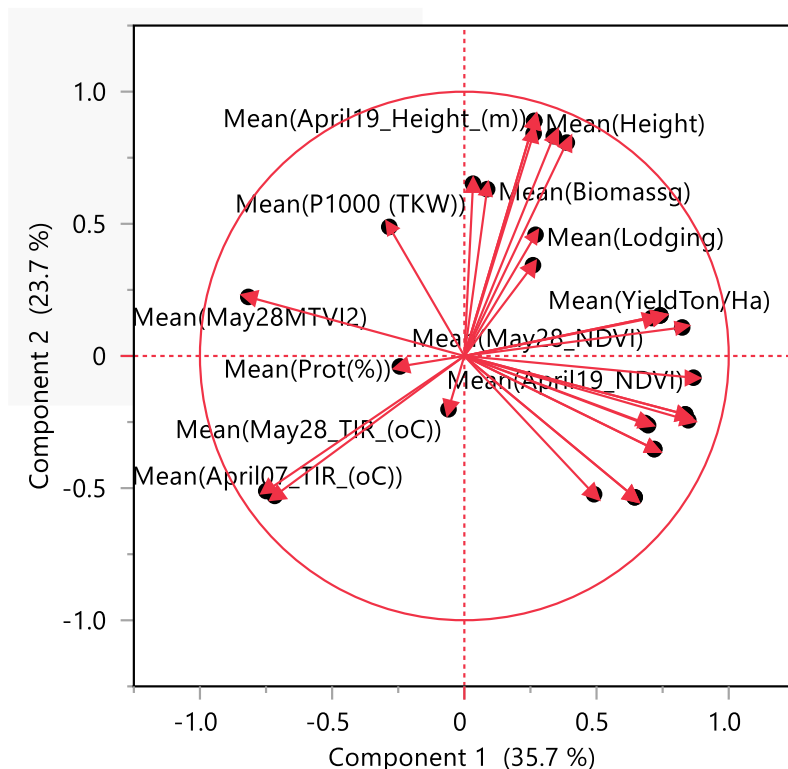


Figure 7. Principal component analysis of yield bioindicators under rainfed conditions of 22 wheat varieties (widely grown in Spain and France).

Under rainfed conditions vegetation indexes (NDVI at anthesis with positive contributions to grain yield) and canopy temperature (at around anthesis with negative contributions to yield) were the most significant traits correlating with grain yield (see Table 4 and Figure 7).

It is concluded that major bioindicators of drought tolerance under Mediterranean conditions are NDVI and Canopy temperature at around anthesis.

### 3.2 Identification of drought tolerant wheat varieties in Spain and France

Multi-factorial analysis of variance for Country (Spain and France), variety (22 wheat varieties) and water regime (irrigated and rainfed) showed significant effects together with all interactions except Country\*Var\*Water (see Table 4). The best varieties in both water regimes were Marcopolo, Complice and Rubisko (Table 5). The most drought tolerant varieties were Complice, Winner and Rubisko (Table 6) with lower Canopy temperatures at anthesis, higher NDVI and higher crop transpiration, early to medium heading dates.



Table 4. Analysis of Variance across countries, varieties and water treatments.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
COUNTRY	1	1	318.20802	453.5517	<.0001*
VAR	21	21	65.79518	4.4657	<.0001*
WATER	1	1	177.26481	252.6610	<.0001*
COUNTRY*VAR	21	21	37.54335	2.5482	0.0005*
COUNTRY*WATER	1	1	6.68683	9.5309	0.0023*
VAR*WATER	21	21	39.24975	2.6640	0.0003*
COUNTRY*VAR*WATER	21	21	18.84655	1.2792	0.1945

Table 5. Mean separation test of varieties across countries and water treatments.

Level		Least Sq Mean
MARCOPOLO	A	10.997913
COMPLICE	A	10.953432
RUBISKO	A B	10.788655
WINNER	A B C	10.649855
UNIK	A B C D	10.433210
SEPIA	A B C D	10.426063
SOLEHIO	A B C D E	10.304762
FANTOMAS	A B C D E	10.298977
RIMBAUD	A B C D E	10.288635
ORLOGE	A B C D E	10.204641
CHAMBO	A B C D E	10.175622
RGT SOMONTANO	A B C D E	10.166959
FILON	A B C D E	10.159090
PORTICCIO	A B C D E	10.085854
LG ABSALON	A B C D E	10.015564
PASTORAL	A B C D E	9.899896
RGT QUIRIKO	A B C D E	9.779087
RGT ALTAVISTA	B C D E	9.655793
CHEVIGNON	C D E	9.417215
RGT ALGORITMO	D E	9.344108
APACHE	D E	9.339816
NOGAL	E	9.175103



Table 6. Mean separation test of wheat varieties under rainfed conditions in Spain and France. \* Wheat variety Winner showed the highest transpiration rate and \*\* RGT Altavista the lowest.

Level	Spain Rainfed		Level	France Rainfed	
	YIELD Tha-1			YIELD Tha-1	
COMPLICE	12.0	A	RUBISKO	8.7	A
WINNER	11.9	A*	COMPLICE	8.6	A
RUBISKO	11.6	A	PASTORAL	8.6	A
MARCOPOLO	11.3	AB	CHEVIGNON	8.5	A
RIMBAUD	11.2	AB	ORLOGE	8.4	A
FILON	11.1	AB	RGT SOMONTANO	8.4	A
ORLOGE	10.9	AB	WINNER	8.3	A
SOLEHIO	10.9	AB	LG ABSALON	8.3	A
LG ABSALON	10.6	ABC	FILON	8.2	A
UNIK	10.6	ABC	SOLEHIO	8.2	A
PASTORAL	10.5	ABC	SEPIA	8.1	A
FANTOMAS	10.5	ABC	MARCOPOLO	8.1	A
RGT SOMONTANO	10.4	ABC	FANTOMAS	8.0	A
CHEVIGNON	10.3	ABC	RIMBAUD	7.9	A
PORTICCIO	10.3	ABC	RGT ALGORITMO	7.9	A
CHAMBO	10.2	ABC	CHAMBO	7.8	A
SEPIA	10.2	ABC	APACHE	7.8	A
RGT QUIRIKO	10.1	ABC	UNIK	7.7	A
APACHE	10.0	ABC	RGT QUIRIKO	7.6	A
NOGAL	9.9	ABC	RGT ALTAVISTA	7.4	A
RGT ALGORITMO	9.0	BC	PORTICCIO	7.3	A
RGT ALTAVISTA	8.5	C**	NOGAL	6.9	A

## 4 Conclusion

The most promising bioindicators of drought tolerance were NDVI, Canopy temperature and heading date. NDVI and canopy temperature share the advantage to be easily measured in field using sensors carried out by various high throughput phenotyping tools (UAV, buggies...). These are two interesting indicators to potentially integrate in Plant Variety Testing for drought tolerance. Consequently, they are usable in large field experiments for breeders, agronomists, and geneticists. They could also provide some informative assessments in farmers' fields. By using the



root systems variables assessed in trials, we'll be able to understand more in depth the correlations between these bioindicators and the corresponding architecture and physiological pathways.

