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1 Potential of high-spectral resolution for field  
2 phenotyping in plant breeding: application to maize  
3 under water stress

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10 **Abstract**

11 Spectroscopy is today and for two decades strongly used in many fields  
12 (pharmacy, agriculture, process, medicine...). This use in a very large number  
13 of applications is linked to the great spectral richness of the measurement  
14 and therefore to the large amount of accessible chemical information. For  
15 plant breeding, spectral reflectance in the visible and near-infrared range  
16 (VIS-NIR) embeds a lot of information about vegetation (pigments, struc-  
17 ture, water, etc.). Discriminatory power between genotypes can be greatly  
18 improved by using high spectral resolution. NIR spectroscopy is still limited  
19 in the field for phenotyping compared to existing imaging solutions that are  
20 easier to implement.

21 In this study, we will address the potential of high spectral resolution data  
22 by using NIR spectroscopy to describe phenotypic responses of maize geno-  
23 types to water stress. To that end, data acquired following an experimental  
24 design with water-deficient environment are processed using an analysis of

25 variance method adapted to multivariate data called REP-ASCA. For each  
26 factor, this method gives its significance, the loadings describing the im-  
27 pacted spectral regions and the scores to classify observations. For a date  
28 with proven water stress, the treatment and genotype factors and the inter-  
29 action term are significant with a p-value threshold at 0.05. Treatment term  
30 loadings highlight the spectral regions impacted by the change in irrigation  
31 while those of the genotype factor allows to group genotypes according to the  
32 yield potential regardless the irrigation. The interaction term loadings are  
33 used as a phenotyping trait related to water stress response. Based on this  
34 signature, tolerant genotypes are differentiated from sensitive genotypes ac-  
35 cording to a ranking based on final yield ( $R = 0.81$ ). This spectral signature  
36 was then applied to another environment with a moderate water deficit. For  
37 most genotypes, we were able to recover the ranking previously established  
38 by the stressed environment ( $R = 0.60$ ).

39 *Keywords:*

40 Plant Breeding, Phenotyping, Spectroscopy, REP-ASCA, Drought, Water  
41 Stress, Maize, Multivariate Data, Chemometrics

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## 42 **1. Introduction**

43 In an agricultural context, drought is defined as any lack of water that  
44 prevents crops to reach their yield potential or that affects quality of har-  
45 vested products. This stress induces changes in yield and quality of many  
46 crops around the world. Periods of drought are increasingly frequent and  
47 severe and require the development of new genotypes adapted to extreme  
48 water stress conditions [1, 2, 3].

49 Furthermore, stress will have a different impact depending on the devel-  
50 opment stage at which it occurs [4, 5, 6]. For example, water stress will have  
51 less impact on the plant when it occurs during the vegetative stage than the  
52 flowering stage. In order to select genotypes according to their ability to  
53 respond to this stress, it is necessary to have rapid and efficient phenotyping  
54 tools to characterize plants in many situations. Therefore, efforts to improve  
55 high-throughput phenotyping tools must be made [7, 8].

56 Technologies with high spectral resolution are ideal for plant phenotyp-  
57 ing [9, 10, 11]. High spectral resolution in the visible and near-infrared range  
58 (VIS-NIR) provides rich information on bio-chemical content and plant struc-  
59 ture [12]. Indeed, the use of highly resolved spectral data has shown its in-  
60 terest in crop monitoring [13, 14, 9] or in early detection of biotic or abiotic  
61 stresses [15, 16, 17]. Additionally, some studies have shown the interest to use  
62 high spectral resolution in plant breeding, particularly for yield prediction  
63 [18, 19].

64 In recent years, technologies tend to jointly increase spectral and spatial  
65 resolution with the use of hyperspectral cameras for phenotyping crops [20].  
66 These technologies still present operational constraints for agricultural ap-  
67 plications (cost, weight, ease of use or acquisition time). On the other hand,  
68 less expensive solutions such as micro-spectrometers provide a high spectral  
69 resolution at the expense of spatial resolution [21].

70 However, analyzing spectral data acquired on vegetation is challenging,  
71 particularly in plant breeding where the objective is to compare genotypes  
72 with similar behaviors. Besides, data collected on several experimental sites  
73 with different agronomic and pedoclimatic conditions brings an additional

74 difficulty. Indeed, the environment has a strong influence on the expres-  
75 sion of a large quantity of genes [22, 23] and hence on the phenotypic traits  
76 measured. These effects must be taken into account to ensure the represen-  
77 tativeness of measured phenotypic traits regardless of the environment and  
78 the development state of the plant.

79 In chemometrics, methods can be used to exploit spectral data [24, 25].  
80 The choice of the method to be used depends on the objective of analysis  
81 of the spectral data. We can dissociate four cases of use of spectral data in  
82 the order of the most commonly used. First, many applications of NIR spec-  
83 troscopy are aimed at predicting biochemical variables from spectra. In this  
84 case, the best known and most commonly used method is partial least squares  
85 regression (PLSR) [26]. A second use case is the use of spectra to predict  
86 a class which combines partial least square (PLS) and discriminant analy-  
87 sis (DA) [27]. A third use case is the exploration of spectral data through  
88 unsupervised approaches such as Principal Component Analysis (PCA) [28].  
89 The last case, very little used but nevertheless very useful when performing  
90 experiments, is the use of methods for the analysis of variance of spectral  
91 data.

92 Experimental design, often called Design of Experiments (DoE) [29] and  
93 ANalysis Of VAriance (ANOVA)[30] are commonly used in plant breeding  
94 [31]. On the one hand, DoE is a way of organizing experiments so that they  
95 allow genotypes to be tested optimally against several variability factors. On  
96 the other hand, the analysis of variance is a statistical method to separate  
97 different factors of variability.

98 Usually, methods of analysis of variance are not adapted to spectral data.

99 Indeed, variables are highly correlated with each other and normality assump-  
100 tions are not satisfied [32]. The most commonly used method is Analysis of  
101 variance - Simultaneous Component Analysis (ASCA) [33] which is widely  
102 used in data analysis of laboratory experiments.

103 In the case of spectral measurements made in the field, errors related to  
104 the lack of repeatability of the measurements may affect analysis conclusions.  
105 An analysis of variance method called Reduction of repeatability error for  
106 Analysis of variance-Simultaneous Component Analysis (REP-ASCA) [34]  
107 has recently been developed to reduce this repeatability error and to describe  
108 identified factors. REP-ASCA also highlights spectral regions associated with  
109 each factor and reduces uncontrolled effects (such as leaf angle, sunlight,  
110 temperature, ...).

111 In this study, we are assessing the potential of high spectral resolution  
112 to phenotype different maize genotypes, specifically in the context of adap-  
113 tation to drought. We will show how REP-ASCA approach applied to spec-  
114 tral data collected in the field can identify spectral signatures as phenotypic  
115 traits to describe genotype responses to water stress. For this purpose, two  
116 experimental campaigns were set up to study the response of different maize  
117 genotypes.

118 In this article, we will first describe the two experimental campaigns.  
119 Then, REP-ASCA will be used to analyze spectra collected in the highest  
120 drought environment to obtain spectral signatures related to the different  
121 studied factors. Finally, the relevance of these signatures will be discussed  
122 in the second environment.

123 **2. Materials and methods**

124 *2.1. Field experiments*

125 Two experimental campaigns were conducted on Limagrain experimental  
126 sites. The first one took place in 2017 in Aubiat (France) and the second one  
127 in 2018 in Nérac (France). The same experimental design was used for both  
128 campaigns (Table 1).

Table 1: Summary of the experimental conditions in 2017 and 2018.

Year	Location	Irrigation	Number of genotypes	Number of replicates	Plant density
2017	Aubiat	Irrigated	10 Genotypes	2 Replicates	9.6 Plants/m <sup>2</sup>
2017	Aubiat	Rainfed	10 Genotypes	2 Replicates	8.5 Plants/m <sup>2</sup>
2018	Nérac	Irrigated	10 Genotypes	2 Replicates	8.5 Plants/m <sup>2</sup>
2018	Nérac	Rainfed	10 Genotypes	2 Replicates	8.5 Plants/m <sup>2</sup>

129 This design was organized in a complete and balanced randomized block  
130 design to compare ten commercial genotypes under two irrigation conditions.  
131 The irrigation conditions or treatments were as follows: one under optimal  
132 irrigation conditions with irrigation triggering and another without irrigation,  
133 i.e. rainfed.

134 Ten commercial genotypes, chosen to have contrasting tolerances to water  
135 stress, were identified by letters from A to J. Genotypes A and C were known  
136 to be highly sensitive while genotypes B, E and F were known to be tolerant.  
137 The remaining genotypes (D, G, H, I and J) were selected for their average  
138 behavior to water stress. These behaviors differed according to water stress  
139 severity. This experimental design had two replicates (two micro-plots) of  
140 the same genotype per treatment. For both experimental campaigns, forty  
141 micro-plots were sowed in four rows and 6m-long each. In Nérac (2018)

142 the density was 8.5 plants/m<sup>2</sup> for both treatments. In Aubiat (2017), the  
143 densities were 8.5 plants/m<sup>2</sup> and 9.6 plants/m<sup>2</sup> respectively under rainfed  
144 and optimal irrigation conditions.

#### 145 *2.1.1. Meteorological data*

146 Temperature, sunshine, rainfall and humidity were locally measured using  
147 meteorological stations installed in the fields. In addition, tensiometers (Wa-  
148 termark probes) were installed to describe water reserves for both irrigation  
149 conditions. Weather data were collected every hour whereas tensiometric  
150 data were collected four times a day.

151 Besides, vapour pressure deficit (VPD) was used to describe the evapo-  
152 rative demand [35].

#### 153 *2.1.2. Agronomic data*

154 Several agronomic data were collected during plant growing and at har-  
155 vest: flowering dates, plants counts, grain yield and moisture. All plots were  
156 harvested with a twin-plot combine DP4000 BAURAL, collecting grain yield  
157 and grain moisture. Grain moisture was measured by NIR (POLYTECH in-  
158 strument). Grain yield was determined by weighing all the grains in each plot,  
159 adjusted to 15% grain moisture and converted to tons per hectare (q/ha),  
160 considering the differences in the plot size across trials. Flowering dates were  
161 measured as the number of days from June 1st, to the time when silks had  
162 emerged on 50% of the plants in a plot. Plant counts were measured as the  
163 numbers of plants after emergence. The micro-plot qualitative scorings were  
164 measured throughout the period of the experiment in order to eliminate plots  
165 where issues other than genotypic related occurred.



166 *2.1.3. Spectral measurements*

167 Spectral measurements were performed with a device designed specifically  
168 for this study to collect data over the maize canopy.

169 This device was designed to address the UAV specifications (Fig. 1).  
170 It included a spectrometer (MMS1, Carl Zeiss Spectroscopy GmbH, Ger-  
171 many) with 256 spectral bands defined in a spectral range from 310 nm  
172 to 1100 nm and a spectral resolution of 3 nm, an integrating sphere (30-  
173 REFL, AvaSphere), a microcomputer (pico-ITX, VIA) and three optical  
174 fibers (550  $\mu\text{m}$ -core diameter, numerical aperture of 0.22, Sedi-ATI). Each  
175 spectrometer measurement  $S$  was normalized by the corresponding integra-  
176 tion time  $t_s$  to have the signal  $I$  as follows:

$$I = S/t_s \quad (1)$$

177 The incident sunlight  $I_0$  was measured with the integrating sphere con-  
178 nected to the spectrometer. Light reflected by the vegetation  $I_v$  was mea-  
179 sured at a distance of approximately 1 m above the canopy corresponding to  
180 an imprint of a 16 cm-diameter circle. Dark measurements  $I_d$  were performed  
181 with a shutter in front of the spectrometer to subtract electronic noise from  
182 signals. A manual switch was used to toggle between the measurement of  
183 incident radiation and canopy radiation with the same spectrometer (Fig.  
184 1).

185 Finally, reflectance spectrum was obtained according to the following  
186 equation:

$$R = \frac{I_v - I_d}{I_0 - I_d} \quad (2)$$

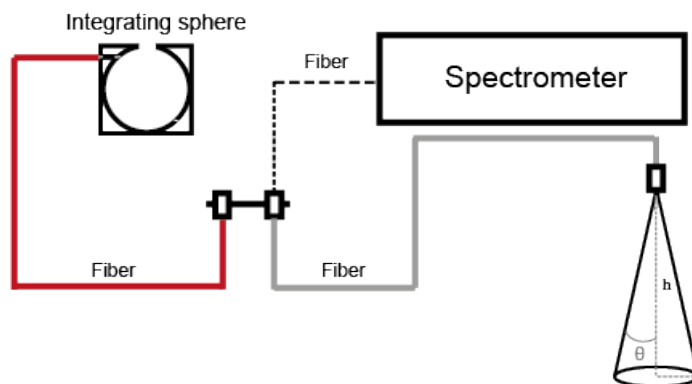


Figure 1: Diagram of the acquisition system; A manual switch was connected to the spectrometer (dot-line), to the integrating sphere (red) and a fiber (grey) for measuring light from vegetation with a numerical aperture ( $\theta = 0.22$ )

187 A wheelbarrow as support vector (Fig. 2) was designed to embed the  
 188 spectral acquisition system. The height being adjustable, it was set at the  
 189 beginning of each acquisition date at a height of approximately 1 meter from  
 190 the top of the canopy.

#### 191 2.1.4. Protocol for spectral field measurements

192 Spectral measurements were performed on 6 dates per year using the  
 193 device presented in section 2.1.3. Measurements were made on one of the two  
 194 central rows of each micro-plot to avoid border effects from the two adjacent  
 195 genotypes. Acquisition path was defined according to the orientation of the  
 196 field and the acquisition time in order to minimize shadows caused by the  
 197 measuring device. A reference measurement with the integrating sphere was  
 198 acquired systematically for each measurement on vegetation. At the end, 12  
 199 spectral reflectances were obtained per micro-plot and 480 spectra over the  
 200 whole field for each acquisition date.



Figure 2: Image of the wheelbarrow embedding the spectral acquisition system, next to a car to visualize the height of the device.

201 *2.2. Data analysis*

202 *2.2.1. Theory of REP-ASCA*

203 REP-ASCA method [34] is used to analyze multivariate data associated  
 204 with a design of experiments. The specificity of REP-ASCA is to reduce  
 205 effects due to a lack of repeatability of measurements. As detail in [34],  
 206 REP-ASCA requires a multivariate data set represented by a matrix  $\mathbf{X}$  for the  
 207 analysis of variance and a data set represented by a matrix  $\mathbf{W}$  to describe the  
 208 repeatability error. In this matrix notation, rows correspond to observations  
 209 and columns to spectral bands. The matrix  $\mathbf{W}$  contains centered measures

210 per packet of repeated measurements and carries only the information related  
 211 to the repeatability error. The centered measures per packet are obtained  
 212 by removing, for each spectrum, the average spectrum of the corresponding  
 213 packet. This operation provides a set of observations containing within-  
 214 variance.

215 REP-ASCA approach aims to define first a subspace representative of  
 216 the repeatability error and then to perform an orthogonal projection of the  
 217 multivariate data  $\mathbf{X}$  to remove this subspace, as detailed in [36]. To define  
 218 this repeatability error subspace, a Principal Component Analysis (PCA) is  
 219 performed on  $\mathbf{W}$  to give principal components. The choice of the first  $k$   
 220 components according to criteria specific to the dataset to be realized allows  
 221 to define this vector subspace named here  $\mathbf{D}$ .

222 The orthogonal projection is then performed to obtain a corrected matrix  
 223  $\mathbf{X}_\perp$ . This projection can be written as follows:

$$\mathbf{X}_\perp = \mathbf{X}(\mathbf{I} - \mathbf{D}\mathbf{D}^t) \quad (3)$$

### 224 2.2.2. Application of REP-ASCA

225 The algorithm REP-ASCA , [available at github.com/RYCKEWAERT/REP-](https://github.com/RYCKEWAERT/REP-ASCA)  
 226 [ASCA](https://github.com/RYCKEWAERT/REP-ASCA), has been implemented with Matlab R2015b (The Mathworks, Natick,  
 227 MA, USA).

228 No pretreatment was performed on spectra. Data acquired on the most  
 229 representative date of a stressed environment were used for REP-ASCA. This  
 230 dataset of 480 acquired spectra was randomly split into 2 datasets to form  
 231 the two matrices  $\mathbf{X}$  and  $\mathbf{W}$  required for REP-ASCA.  $\mathbf{X}$  was thus composed  
 232 of 2/3 of the measurements, i.e. 320 spectra. The remaining measurements

233 were centered in packets of repeated measures to form the matrix  $\mathbf{W}$ .  $\mathbf{W}$   
 234 was thus made up of 160 spectra.

235 The classical ASCA method [33] was then applied to the corrected spec-  
 236 tral data  $\mathbf{X}_\perp$ , obtained after orthogonalization (eq. 3). As all analysis of  
 237 variance of multivariate data [33, 34],  $\mathbf{X}_\perp$  was decomposed into matrices  
 238 according to the variances associated with the effects studied and their inter-  
 239 actions, namely here genotype, treatment effects and genotype-environment  
 240 interaction. This decomposition is written as follows:

$$\mathbf{X}_\perp = \mu + \mathbf{X}_G + \mathbf{X}_T + \mathbf{X}_{G \times T} + \mathbf{E} \quad (4)$$

241 Where  $\mu$  denotes the overall mean matrix of  $\mathbf{X}_\perp$ . Here,  $\mathbf{X}_G$ ,  $\mathbf{X}_T$  and  
 242  $\mathbf{X}_{G \times T}$  are matrices corresponding to the genotype, treatment and genotype  
 243 x treatment interaction effects, respectively. The matrix  $\mathbf{E}$  represents the  
 244 residuals. Each spectrum (line) from  $\mathbf{X}$  is then decomposed as a sequence  
 245 of average spectra per modality. For example, the matrix  $\mathbf{X}_T$  contains for  
 246 each row corresponding to an observation, either the average spectrum of the  
 247 irrigated condition or the average spectrum of the non-irrigated condition.

248 The second step is to perform a permutation test to compute p-values  
 249 to determine if the factors are significant. The last step is to reduce the  
 250 dimensionality of each significant factor by simultaneous component analysis  
 251 (SCA). For a given factor  $i$ , the corresponding matrix  $X_i$  is then written as :

$$\mathbf{X}_i = \mathbf{T}_i \mathbf{P}_i^t + \mathbf{R}_i \quad (5)$$

252 Where  $\mathbf{P}_i$  is the matrix containing loadings of the principal components  
 253 (PC),  $\mathbf{T}_i$  the scores and  $\mathbf{R}_i$  the residuals of the SCA.

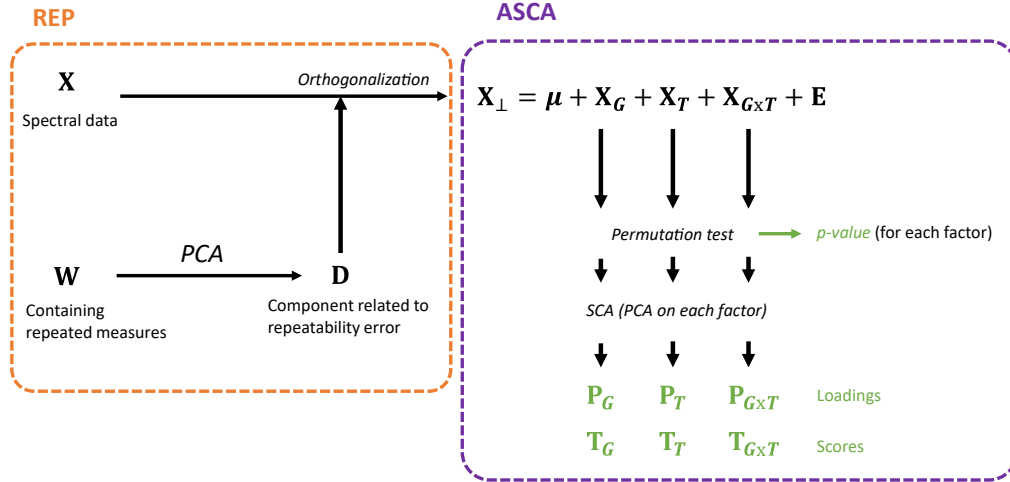


Figure 3: Flow chart to summarize REP-ASCA method.

254 **3. Results and discussion**

255 *3.1. Description of experimental campaigns*

256 *3.1.1. Climatic conditions*

257 Water stress results from a combination of a lack of water in the soil, high  
 258 evaporative demand and high temperatures. The assessment of the water  
 259 stress intensity on the micro-plots and for each date was performed using  
 260 the available meteorological, i.e. measurements of maximum temperatures,  
 261 tensiometry and vapour pressure deficit (VPD).

262 All climatic variables at the acquisition dates are providing in the table  
 263 2). In the first column, the acquisition dates are numbered from 1 to 6 in  
 264 chronological order for each year for descriptive convenience.

265 For all other dates and with differences that do not exceed 5°C between

266  $T_{max,d}$  and  $T_{max,-3d}$ , Over this period, there were therefore no abrupt changes  
267 in temperature.

268 Tensions at the acquisition dates for the two treatments and differences  
269 between treatments are given in table 2. A tension value represents the force  
270 required to extract water from the soil, so the higher the tension, the drier  
271 the soil. In Aubiat (2017), tension values for both treatments are very low,  
272 with maximum tensions being reached on the third date with values equal  
273 to 73.42 and 50.08 cbar for the rainfed and irrigated treatments respectively.  
274 At this date, the difference  $\delta P$  is maximal with a value equal to 23.34 cbar.

275 In Nérac (2018), the tension value of the rainfed plot is high with a  
276 maximum value equal to 231.5 cbar at the third date. For irrigated plot,  
277 the tension value increases to 78.5 cbar that is already higher than all value  
278 in Aubiat (2017), even in irrigated condition. On this date, a difference of  
279 153 cbar is then observed for tension values between irrigated and rainfed  
280 conditions and decreases on the following dates.

281 In Aubiat (2017), tensiometry and VPD values are very low. In addition,  
282 tension differential between irrigated and non-irrigated plots is very low due  
283 to the presence of significant rainfall events. These two plots are therefore  
284 under optimal water reserve conditions even if a slight differential appears  
285 between the two plots.

286 In Nérac (2018), the tensiometric value is high for the rainfed condition,  
287 combined with average evaporative demand and high temperatures. The  
288 values obtained in Nérac (2018) are those expected for trials under water  
289 stress conditions.

290 Although the maximum temperatures recorded in 2017 and 2018 are al-

291 most identical, rainfall and evaporative demand are different between these  
292 two environments. Aubiat (2017) has a low value for tension difference be-  
293 tween treatments corresponding to low water stress. On the other hand,  
294 Nérac (2018) is the environment with the highest water stress on the rainfed  
295 condition.

296 At the measurement dates, plant transpiration is higher in Nérac (2018)  
297 than in Aubiat (2017) where rainy episodes occurred. High values correspond  
298 to a high evaporative demand. In this situation, the air is dry and increases  
299 plant transpiration.

300 These findings are confirmed by the tensions values. We therefore select  
301 the most contrasted dates in terms of drought intensity with the maximum  
302 differences in tension values between the two treatments.

303 On all available acquisition dates, the third date (07/30/18) of Nerac  
304 (2018) is the acquisition date when the water stress is the most severe. Spec-  
305 tral data acquired on this date is used for the REP-ASCA study. The third  
306 date (02/08/17) of Aubiat (2017) is the acquisition date with a maximal  
307 difference between treatments. This date is used as a second environment  
308 with low water stress. These two dates correspond to the grain filling stage  
309 which is one of the most sensitive stages to water stress. Indeed, at this date  
310 drought has an impact on grain filling and therefore on the thousand kernel  
311 weight (TKW) [37, 5].

### 312 3.1.2. Agronomic variables

313 The table 3 shows, by treatment, means and standard deviations of yield  
314 values obtained at harvest.

315 In 2017, average yield values for irrigated and rainfed conditions are high,



Table 2: Description of the climatic conditions for each acquisition date: Maximum temperatures  $T_{max,d}$ (°C) for the acquisition dates and averaged over the 3 previous days of the acquisition dates  $T_{max,-3d}$  (°C), rainfed cumulated during the 3 days preceding the acquisition date  $R_{-3d}$ (mm), vapour pressure deficit (VPD)(kPA), tensiometry P(cbar) for both treatment and difference between treatments  $\delta P$ (cbar).

N°	Location	Date	$T_{max,d}$ (°C)	$T_{max,-3d}$ (°C)	$R_{-3d}$ (mm)	VPD (kPA)	P (cbar)		$\delta P$ (cbar)
							rainfed	irrigated	
1	Aubiat	19/06/17	34.5	30.0	6.75	1.53	5.83	5.83	0
2	Aubiat	19/07/17	28.0	33.0	6.75	0.89	13.08	11.41	1.67
3	Aubiat	02/08/17	33.5	31.0	3.5	0.95	73.42	50.08	23.34
4	Aubiat	17/08/17	29.5	30.5	0.25	0.80	43.92	26.01	17.91
5	Aubiat	01/09/17	19.5	34.5	19.8	0.31	2.92	1.42	1.5
6	Aubiat	28/09/17	25.0	24.0	18.3	0.47	1.42	1.42	0
1	Nérac	26/06/18	31.6	29.1	0	1.60	x	x	x
2	Nérac	10/07/18	28.8	31.1	0.6	1.19	145.08	x	<145.08
3	Nérac	30/07/18	30.8	34.2	0.4	1.25	231.5	78.5	153
4	Nérac	20/08/18	29.5	29.9	0.2	1.30	145.17	86.67	58.5
5	Nérac	27/08/18	29.8	30.6	4.0	1.23	229.5	121	108.5
6	Nérac	10/09/18	26.9	29.4	11.8	0.83	239	168	71

316 with 133.5 q/ha and 116.2 q/ha respectively. In 2018, average yield values  
317 are much lower with 104.42 q/ha for irrigated condition and 85.46 q/ha for  
318 rainfed condition. Standard deviations are of the same order of magnitude  
319 for all the experimental campaigns with values between 9 and 13 q/ha.

320 Obtaining lower yield values in rainfed condition compared to irrigated  
321 condition was expected. In 2017, yield values in both conditions are high.  
322 Indeed, as stress is very low (Tab. 2), yield values in rainfed condition remain  
323 high (Tab. 3).

Table 3: Average and standard deviation of the yield obtained at harvest for both irrigation conditions and for each environment.

Year	Yield (q/ha)			
	Irrigated		Rainfed	
	Average	Standard deviation	Average	Standard deviation
2017	133.5	9.36	116.2	10.85
2018	104.42	12.48	85.46	9.12

324 *3.1.3. Genotypes classification based on yield*

325 In our study, the percentages of yield loss between two irrigation condi-  
 326 tions are used to describe genotypes responses to water stress. Thus, for a  
 327 given genotype, the lower the percentage of loss between the two irrigation  
 328 conditions, the less sensitive the genotype will be to water stress. On the  
 329 contrary, a high percentage of loss between the two irrigation conditions will  
 330 show a high sensitivity of the genotype to water stress. The yield value of  
 331 the irrigated condition is also important. Indeed, it shows the yield potential  
 332 of genotypes under optimal irrigation conditions.

333 In general, several criteria are needed to characterize resistance to water  
 334 stress [38]. Climatic variables help to put yield values obtained into con-  
 335 text. The 2017 environment does not correspond to a stressed environment.  
 336 Therefore, it is difficult to assess genotype sensitivity to irrigation conditions  
 337 in this particular environment. The year 2018 was therefore used to verify  
 338 that the genotypes were correctly classified according to their sensitivity to  
 339 water stress.

340 For each genotype, yield values and the percentage of yield loss between  
 341 the two irrigation conditions for 2018 are given in table 4.

Table 4: Average yield per treatment and percentage losses for each genotype for 2018.

Genotype	Yield 2018 (q/ha)		
	Irrigated	rainfed	Loss (%)
A	103.22	83.17	19.42
B	105.85	97.01	8.34
C	115.39	88.92	22.94
D	100.82	93.30	7.45
E	78.39	77.13	1.61
F	103.63	92.49	10.74
G	118.55	86.65	26.91
H	104.16	76.29	26.75
I	114.51	81.64	28.70
J	99.68	77.99	21.75

342 In irrigated conditions, some genotypes have very high yields such as  
 343 genotypes C and G with average values of 115.39 q/ha and 118.55 q/ha  
 344 respectively. Others have very low yield values such as genotypes E and  
 345 J with average values equal to 78.39 q/ha and 99.68 q/ha respectively. In  
 346 rainfed conditions, genotypes with high yield values are genotypes B and D  
 347 with respectively 97.01 q/ha and 93.30 q/ha. Genotypes E and J have the  
 348 lowest yields with values of 77.13 q/ha and 77.99 q/ha respectively.

349 Genotypes that have a low percentage of losses between irrigated and  
 350 rainfed conditions are genotypes B, D and E with a loss of 8.34%, 7.45%  
 351 and 1.61% respectively. Very sensitive genotypes are genotypes A, C, G, H,  
 352 I and J with losses of 19.42%, 22.94%, 26.91%, 26.75%, 28.70% and 21.75%  
 353 respectively.

354 Based on agronomic data, this analysis allows us to establish two classes

355 of genotypes: the first class includes tolerant genotypes (B, D, E and F). For  
356 these genotypes, a change in irrigation has little impact on yield. The second  
357 class is composed of sensitive genotypes (A, C, G, H, I and J). For these  
358 genotypes, yield is strongly impacted by the change in irrigation. Genotype  
359 E is atypical as it seems to reach its yield potential even in the absence of  
360 water.

### 361 3.2. Reduction of repeatability errors with REP-ASCA

#### 362 3.2.1. Descriptions of the spectral measures

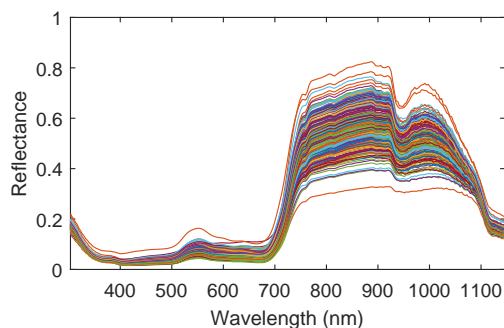


Figure 4: Spectral data for the chosen date (30/07/18 - Nérac).

363 Figure 4 shows spectra acquired in 2018 corresponding to characteristic  
364 spectra of vegetation: presence of specific hollows at 450 nm and 650 nm  
365 related to chlorophyll content; the slope between 700 nm and 800 nm corre-  
366 sponding to the red-edge; a large reflected part related to internal structure  
367 beyond 750 nm and a hollow at 950-980 nm related to water content. It  
368 can be seen that these spectra are affected by a systematic variation effect  
369 consisting of a vertical translation visible at 400 nm (additive effect) and  
370 a multiplicative effect visible between 800 and 1000 nm. This last effect is  
371 particularly visible when reflectance signal intensity is high.

372 Additive and multiplicative effects are classically observed on vegetation  
 373 spectra [39]. Additive effects are mainly due to variations in angles formed  
 374 between normal at the leaf surface and measurement axis [40] during spectral  
 375 acquisition. Multiplicative effects show variations as a function of wavelength  
 376 and are essentially related to the increase of optical path [40]. Additive and  
 377 multiplicative effects are generally addressed by the application of standard-  
 378 ization methods such as Standard Normal Variate (SNV) [41] and Variable  
 379 Sorting for Normalization (VSN) [42]. However, these pretreatments can  
 380 have deleterious effects on spectra [43, 44, 42]. For this reason, no pretreat-  
 381 ment has been applied here.

382 *3.2.2. Selection of the dimension  $k$  of the detrimental space*

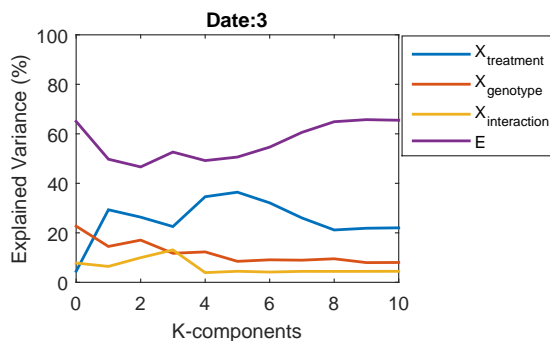


Figure 5: Impact of projections on explained variances for each factor in the analysis of variance on spectral acquired at the chosen date (30/07/18 - Nérac).

383 The objective is to determine the  $k$  dimension of the detrimental subspace  
 384 corresponding to the repeatability errors.  $k$  must be judiciously chosen to  
 385 define the detrimental subspace corresponding to the repeatability error while  
 386 avoiding removing any part of the variance related to a factor of interest.

387 This choice can be driven by various criteria. Here we choose to look at the  
388 impact of the components number  $k$  removed by orthogonal projection on  
389 the variances carried by each factor of the data set  $\mathbf{X}_\perp$ .

390 Figure 5 shows the evolution of the explained percentage variance of the  
391 matrix  $\mathbf{X}_\perp$  for each of factors studied as a function of the number  $k$  of  
392 orthogonal projections performed.

393 Without correction, the percentage of explained variance of the residual  
394 term is very high and reaches 65.01% of the total variance. It is always  
395 higher than other factors regardless of  $k$ . This percentage reaches a minimum  
396 of 46.63% for  $k=2$ . For values greater than 2, this percentage increases  
397 progressively to reach a maximum value of approximately 65% for  $k=10$ .

398 For the treatment factor, the percentage variance explained is 4.45% of  
399 the total variance when no correction is made ( $k=0$ ). After orthogonal  
400 projection according to the first component ( $k=1$ ), this percentage is then  
401 29.32%. It then varies between 22% and 36% for  $k$  ranging between 2 and 10.  
402 The treatment term seems to be strongly impacted by the first component  
403 related to repeatability error. For the genotype factor, the percentage of  
404 variance explained decreases progressively when  $k$  increases from 14.5% when  
405  $k=1$  to 8.03% when  $k=10$ . For the interaction term, the percentage variance  
406 explained increases slightly for  $k=3$  to reach 13.09%. But from 4 projections,  
407 it drops sharply to around 3-4% of the total variance. For a value of  $k$  greater  
408 than or equal to 4, the variance explained for the interaction term no longer  
409 changes. The projection then has a negative effect and removes the variance  
410 related to this factor.  $k$  must therefore be less than 4.

411 We choose a value of  $k$  that minimizes the percentage of residual variance

412 while maximizing those of the factors of interest. The choice was therefore  
 413  $k=2$ .

414 *3.2.3. Loadings of the principal components related to the repeatability error*

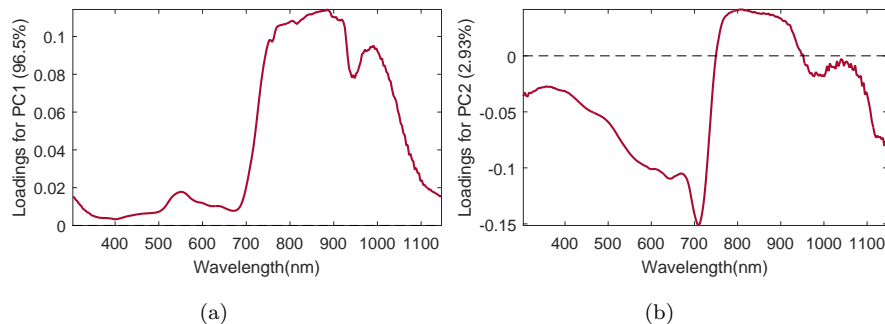


Figure 6: Description of  $\mathbf{W}$ : Loadings of (a) PC1, (b) PC2.

415 Loadings of the first component (PC1) of the matrix  $\mathbf{W}$  are visible in  
 416 figure 6a. They represent 96.5 percent variance in repeatability error. These  
 417 loadings are all positive and are similar to the spectra obtained on vegetation  
 418 (Fig. 4). This corresponds to a systematic variation corresponding with  
 419 the average spectrum. It can therefore be seen that a large part of the  
 420 repeatability error is due to the systematic effects described above (Fig. 4).

421 The loadings of the second principal component (PC2) can be seen in  
 422 figure 6b. These loadings show an opposition between the visible region (300  
 423 - 700 nm) and the near-infrared one (700 - 1100 nm). The repeatability error  
 424 within a micro-plot is expressed here as a difference in the slope between the  
 425 pigment-sensitive visible part and the structure-sensitive near-infrared part  
 426 of the cells. In addition, they show a pronounced negative spike at 709 nm.  
 427 The peak corresponds to a variation in the position of the red-edge and more  
 428 specifically the start of the red-edge [45].

429 It is known [46, 47] that the balance between the visible and near-infrared  
430 parts, as well as the position of the red-edge can vary from leaf to leaf for  
431 the same plant and obviously from one plant to another. It is therefore  
432 not surprising to find these deformations in the repeatability error because  
433 the acquisitions were done at different points in the plants row and indeed  
434 capture the plants under different angles of view.

### 435 3.3. REP-ASCA results

436 The repeatability error of the dataset having been reduced by orthogonal-  
437 ization ( $k=2$ ), ASCA is performed on the corrected dataset  $\mathbf{X}_\perp$ . All factors  
438 in equation 4 are significant with p-values  $< 0.05$  according to permutation  
439 tests. ASCA thus provides loadings and scores for each term of the equation  
440 4.

441 The number of principal components obtained for each term is equal to  
442 the number of levels minus one. The treatment term is a two-level factor  
443 (irrigated/rainfed). So, only one principal component is obtained. For other  
444 terms (genotype and interaction), many principal components are obtained.  
445 However, for simplicity, results on the first two components will be described.

#### 446 3.3.1. Treatment term

447 *Loadings.* The loadings of the principal component are shown in figure 7. In  
448 the visible and near-infrared region, we observe two peaks with the same sign,  
449 separated by a hollow centered at 739 nm. There are also two positive peaks  
450 located at 945 nm and 1040 nm, surrounding a hollow at 970 nm. A positive  
451 slope is also visible between 300 and 420 nm. The hollow located at 739 nm  
452 corresponds to the region of the red-edge and more precisely to its inflection



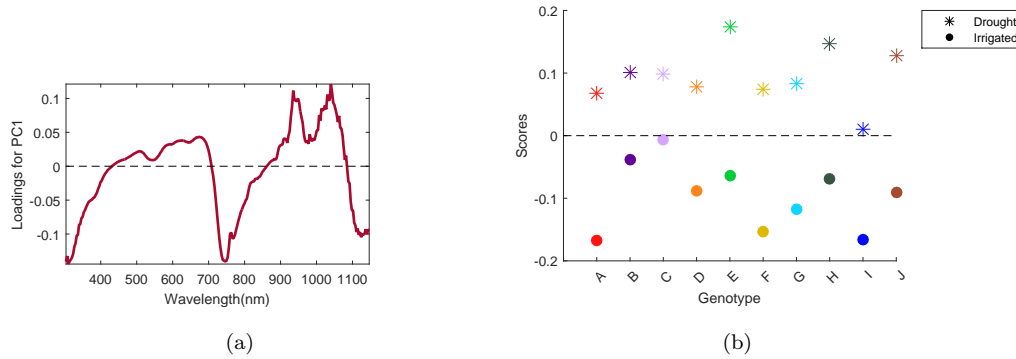


Figure 7: Decomposition of the treatment term : (a) Loadings of the principal component, (b) average scores obtained by genotype.

453 point (maximum of the first derivative). The hollow at 970 nm corresponds  
 454 to the water absorption [48, 49]. The presence of the two peaks at 945 nm  
 455 and 1040 nm express an enlargement of the hollow at 970 nm. The slope  
 456 between 300 and 420 nm shows a difference in absorption in the ultraviolet  
 457 (UV) region between the irrigated and stressed areas. In this region there  
 458 are both the UV-A absorption and the beginning of the region corresponding  
 459 to the UV-B absorption [50].

460 Changes in reflectance in this spectral region can be induced by different  
 461 levels of flavonoids and phenylpropanoids. Indeed, these chemical compo-  
 462 nents absorb strongly in UV radiation [51]. UV absorption directly impacts  
 463 photosynthetic activity, and so, yield [52, 50].

464 *Scores.* Average scores obtained on each genotype are proceeded by project-  
 465 ing their mean spectra onto this principal component. The scores obtained  
 466 for each genotype are presented in figure 7b. The projection corresponds  
 467 to the vector product between a spectrum measured and this component

468 producing a score.

469 The scores obtained in irrigated condition (round symbol) are mainly  
470 negative. Conversely, scores obtained in rainfed condition (star symbol) are  
471 mainly positive. However, genotype I in rainfed conditions and C in irrigated  
472 conditions have scores close to zero. Extreme positive values are also observed  
473 with genotype E (in rainfed condition) and negative with genotypes A and I  
474 in irrigated condition.

475 By jointly analyzing scores and loadings of this component (Fig. 7a),  
476 we can thus deduce information on traits related to genotype in response to  
477 the average behavior per treatment. With negative loadings in 739 nm, a  
478 positive score will correspond to a genotype with a low red-edge slope. And  
479 conversely, with positive loadings between 970 nm, a positive score will have  
480 high reflectance values around the dip at 970 nm. Results are reversed when  
481 scores are of different sign. For example, On the other hand, scores produced  
482 by this component will be negative when the initial spectra show a marked  
483 red-edge and a widening of the absorption peak related to water.

484 In general, genotypes in rainfed conditions show positive scores (Fig. 7b).  
485 Their reflectance spectrum has a less marked inflection point and also a  
486 shallower absorption dip than the same genotypes under irrigated conditions.  
487 These phenomena can express a reduction in leaf water content and changes  
488 in plant metabolism for defense mechanisms regarding drought [53].

489 Indeed, under water stress, the closure of stomata plays a protective role  
490 by reducing water loss and limiting gas exchanges [5, 54]. This stomatal  
491 closure could slow down the photosynthetic process of the plant leading to a  
492 reduction in yield when sporadic stress occurs [55].

493 The study of the treatment term by loading interpretation allow to iden-  
 494 tify the spectral regions impacted by the irrigation change. It is possible to  
 495 identify atypical behaviors with respect to other genotypes tested for a given  
 496 treatment. Genotypes with scores close to zero will not have the same mech-  
 497 anisms as other genotypes when faced a given treatment. On the other hand,  
 498 genotypes with extreme score values will have more pronounced behaviors.

499 *3.3.2. Genotype factor*

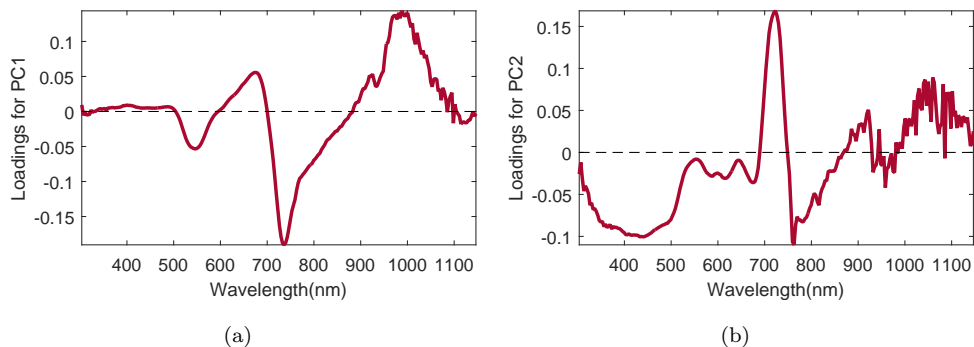


Figure 8: Loadings of the genotype term on (a) PC1, (b) PC2.

500 *Loadings.* Loadings of the first two principal components (PC1 and PC2)  
 501 of the genotype term are shown in figure 8. On PC1 (Fig. 8a), loadings  
 502 in the spectral range 300-500 nm are close to zero. There are two negative  
 503 peaks located at 545 nm and 736 nm and two positive peaks located at  
 504 672 and 980 nm. A slope break at 769 nm occurs in the increasing slope  
 505 visible from 736 to 980 nm. The dips at 545 nm and 736 nm correspond to  
 506 the anthocyanin content and the inflection point of the red-edge, respectively.  
 507 The peaks at 672 nm and 980 nm correspond to the absorption of chlorophyll  
 508 and water, respectively. PC1 will therefore produce negative scores for plants

509 having high chlorophyll and water contents and positive scores for those with  
510 high anthocyanin contents and a less pronounced slope of the red-edge. This  
511 component therefore contrasts genotypes with good photosynthetic activity  
512 (negative scores) and genotypes with a high anthocyanin content that may  
513 result from the presence of various environmental stresses (positive scores)  
514 [56]. The latter therefore will have a lower photosynthetic capacity.

515 The loadings of the second principal component (PC2) (Fig. 8b) have a  
516 large negative part between 350 nm and 500 nm, a peak at 719 nm and a  
517 constant slope from 760 nm to 900 nm. The negative part of 350 nm to 500  
518 nm relates to the region of pigment absorption, particularly carotenoids and  
519 chlorophyll pigments. The peak at 719 nm is the first part of the red-edge.  
520 This component provides additional information to PC1 and also more spe-  
521 cific information on the carotenoid content. These pigments can protect the  
522 photosynthetic system from excess light [57, 58]. Positive scores on PC2 will  
523 reflect lower reflectance at 500 nm and therefore higher carotenoid content.  
524 Genotypes with a high carotenoid content may be selected for their ability  
525 to be tolerant to excess light [59].

526 *Scores.* The plot scores obtained on PC1 and PC2 are shown in figure 9.  
527 According to the previous study of loadings (Fig. 8a and 8b), score value  
528 per genotype is due to differences in red-edge slope and pigments contents  
529 (chlorophylls, anthocyanins and carotenoids). Genotypes B, C, E, H and J  
530 have positive scores on PC1. These genotypes and have therefore a higher  
531 anthocyanin content and a spectral response with a less pronounced red-  
532 edge slope. PC2 mainly separates genotype C and I (negative scores) from  
533 genotypes D, E, F and H (positive scores). Genotypes C and I appear to have

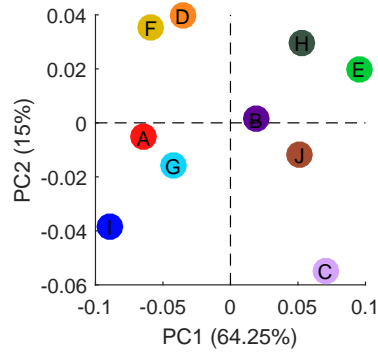


Figure 9: Genotype scores on the first two components (PC1 and PC2) of the genotype term.

534 higher carotenoid levels than genotypes D, F and H. Genotypes A, G, and  
 535 I have a higher chlorophyll content and will therefore favor yield potential  
 536 [59, 60, 61]. Based on these scores, we can describe each genotypes or see  
 537 the grouping of certain genotypes, i.e. genotypes F and D, genotypes A, G  
 538 and I and genotypes E and H.

539 *3.3.3. Interaction term*

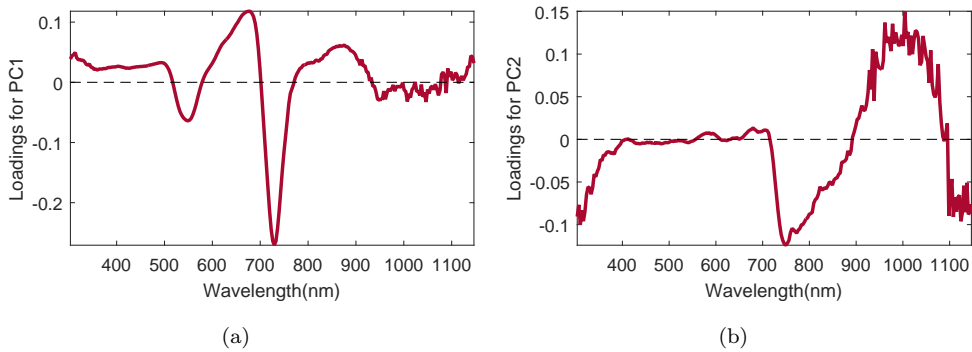


Figure 10: Loadings of the interaction term on: (a) PC1, (b) PC2.

540 *Loadings.* The loadings of the first two components (PC1 and PC2) are  
 541 shown in figure 10. On PC1 (Fig. 10a), a dip is located at 545 nm and  
 542 a hump is located at 672 nm. Another major hollow is located at 729 nm  
 543 and another bump appears from 800 to 922 nm. After 950 nm, loadings  
 544 values are close to zero. The first part of the loadings of this component  
 545 (Fig. 10a) has a similar shape to the loadings of PC1 of the genotype term  
 546 (Fig. 10a) relating to anthocyanin and chlorophyll content.

547 The loadings of PC2 are shown in figure 10b. Surprisingly, loadings values  
 548 are equal to zero between 400 and 700 nm. After a sharp decrease between  
 549 700 to 750 nm, loading values increase and a positive slope is visible from 750  
 550 nm to 1,000 nm. PC2 carries strictly no information on the visible spectral  
 551 region and thus no information on the pigment content. On the other hand,  
 552 it expresses changes in the internal structure of the leaves. Indeed, changes  
 553 such as changes in turgidity and intercellular space can be significant even in  
 554 the presence of very low water stress [62]. In the short term, this stress can  
 555 lead to wilting or leaf curling.

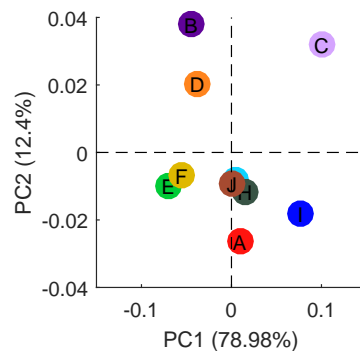


Figure 11: Scores obtained on the first two components PC1, PC2 of the interaction term.

556 *Scores.* Average scores obtained on PC1 and PC2 for each genotype are  
557 presented in figure 11. Some genotypes are grouped together: genotypes E  
558 and F have negative scores on both axes; genotypes G, H and J have scores  
559 close to zero on both axes.

560 According to the loadings of PC1 (Fig. 10a), negative scores for geno-  
561 types E and F are due to their good chlorophyll and anthocyanin content in  
562 irrigated conditions. On this same component, the positioning of genotype  
563 A changes very little between irrigation conditions. For this genotype, the  
564 change in irrigation condition has very little impact in the spectral regions  
565 related to anthocyanins and the position of the red-edge. For this same geno-  
566 type, the score observed on PC2 is strongly negative. The difference between  
567 the two irrigation conditions then seems to be a difference in the desiccation  
568 of internal cells and therefore in the wilting of the leaves. Genotypes B, C  
569 and D have positive scores on PC2. Considering that this component re-  
570 flects internal modification effects of the cells, it can be assumed that these  
571 genotypes do not undergo internal structural degradation under water stress.

#### 572 3.4. *Principal components used as proxies*

573 In this part, we study correlation between scores obtained on the differ-  
574 ent components of the different factors with yield variables measured during  
575 experimental campaigns. This study will enable us to classify genotypes ac-  
576 cording to their tolerance to water stress for the 2018 experimental campaign.  
577 A classification of genotypes for the 2017 experimental campaign will then  
578 be carried out on the basis of the results of the 2018 campaign.

579 *3.4.1. Correlation between scores and agronomic variables*

580 The table 5 shows correlations between the agronomic variables average  
 581 yield for the two irrigation conditions (rainfed/irrigated), the percentage of  
 582 losses and the scores previously calculated for all the terms studied i.e. treat-  
 583 ment, genotype and interaction.

Table 5: Correlation between yield-based agronomic variables and treatment term scores  $\mathbf{T}_T$ ; genotype scores on PC1 ( $\mathbf{T}_{G,1}$ ) and on PC2( $\mathbf{T}_{G,2}$ ); and interaction term scores on PC1( $\mathbf{T}_{G \times T,1}$ ) and PC2 ( $\mathbf{T}_{G \times T,2}$ ).

agronomic variables	$\mathbf{T}_T$	$\mathbf{T}_{G,1}$	$\mathbf{T}_{G,2}$	$\mathbf{T}_{G \times T,1}$	$\mathbf{T}_{G \times T,2}$
Yield (irrigated)	-0.42	-0.48	-0.59	0.69	0.17
Yield (rainfed)	-0.12	-0.34	0.03	-0.2	0.71
Average yield	-0.36	-0.51	-0.38	0.41	0.47
Loss percentage (%)	-0.33	-0.24	-0.59	0.81	-0.34

584 For the treatment term or the genotype term, scores are weakly correlated  
 585 to the agronomic variables with absolute values of the correlation coefficient  
 586 below 0.60.

587 The highest values are obtained for the interaction term: scores on PC1  
 588 ( $\mathbf{T}_{G \times T,1}$ ) are positively correlated (R=0.69) to the yield in irrigated condition.  
 589 Those on PC2 ( $\mathbf{T}_{G \times T,2}$ ) are correlated (R=0.71) to yield in rainfed condition.

590 This confirms that pigment content and steep red-edge slope (Fig. 10a)  
 591 are related to the photosynthetic capacity of the plant and thus its yield  
 592 under optimal irrigation conditions. Under rainfed conditions, variations in  
 593 cell structure (degradation) and plant morphology (drying, wilting, coiling)  
 594 affect yield values.

595 The correlation between the scores on PC1 of the interaction term ( $\mathbf{S}_{G \times T,1}$ )



596 and the percentage of yield loss is highest and equal to 0.81. The study of  
597 loadings (Fig. 10a) indicate that the scores obtained for the different geno-  
598 types express the plant ability to mobilize its photosynthetic system to water  
599 stress. It is therefore possible to retrieve the sensitivity ranking of genotypes  
600 to stress previously established directly from the scores of this component.  
601 Negative scores will therefore correspond to tolerant genotypes and positive  
602 scores to stress-sensitive genotypes.

603 Figure 12, shows the  $\mathbf{S}_{GxT,1}$  scores for all genotypes (Fig. 11) as a func-  
604 tion of the percentage of losses. Indeed, genotypes B, D, E and F are then  
605 classified as tolerant while genotypes J, C, G, H, A and I are classified as  
606 rather sensitive. We can find this classification by using the table 4. All  
607 genotypes with negative score have a percentage of yield loss below 11%. On  
608 the other hand, genotypes with positive scores on this axis have a percentage  
609 of yield loss greater than 20%. Genotype E is very atypical, as it is extremely  
610 tolerant with a yield loss of less than 2% but this genotype has also a very  
611 low yield potential. On the other hand, genotypes B and D are well disso-  
612 ciated from genotypes E and F on the second principal component. These  
613 two pairs are therefore dissociated from each other in terms of leaf structure  
614 (Fig. 10b).

#### 615 3.4.2. Application to another environment

616 Components obtained by REP-ASCA to the data acquired during the  
617 2018 campaign were applied on another environment. The objective is to  
618 study the influence of the environment on the scores obtained. As previously  
619 mentioned, there was no significant water stress on the 2017 experimental  
620 campaign. We will nevertheless apply the principal component of the inter-

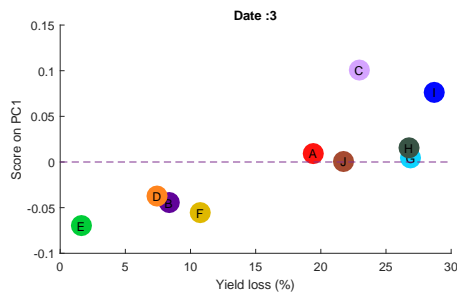


Figure 12: Scores according to the percentages of yield loss.

621 action term obtained in 2018 to data collected in 2017.

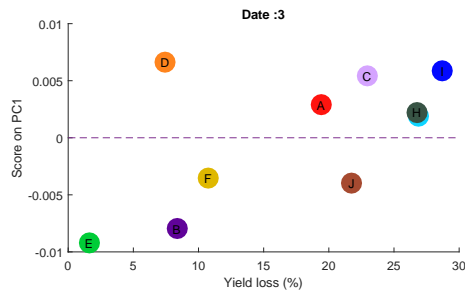


Figure 13: Scores by genotype obtained for 2017 observations based on percentage yield loss in 2018.

622 In 2017, the water reserve of the rainfed part is slightly reduced without  
 623 any significant impact on yield. Percentages of yield loss obtained in 2018 are  
 624 then used to provide a yield-based ranking describing genotypes responses to  
 625 water stress.

626 When comparing genotype rankings obtained for the 2018 (Fig. 12) and  
 627 2017 (Fig. 13) data, we find strong similarities in behavior. Thus, genotypes  
 628 B, E and F scored negative in both environments and are classified as toler-  
 629 ant. Genotypes A, C, G, H and I have positive scores in both environments  
 630 and are classified as sensible to water stress. These similarities are very en-

631 couraging and show that the loadings obtained from an environment with  
632 high water stress can be applied to another environment with lower water  
633 stress.

634 Only genotypes D and J do not meet this classification. It can be hy-  
635 pothesized that these genotypes do not put in place responses identified by  
636 the components on the different factors when the intensity of the stress is  
637 not sufficient. The spectral signatures obtained on these genotypes in this  
638 low water stress environment are not affected in the same way when they are  
639 under intense stress.

640 Although water stress was very limited during the 2017 campaign, we  
641 were able to link the scores obtained on this environment with the ranking  
642 established on a water-stressed environment. The scores obtained on this  
643 component are positively correlated ( $R = 0.60$ ) with their ability to respond  
644 to water stress.

645 This demonstrates that the method described is appropriate to identify  
646 differential responses of genotypes to water stress even in a situation where  
647 this stress is ad hoc, without a major impact on yield. This approach offers  
648 a promising solution for phenotyping a larger number of genotypes for their  
649 drought tolerance without implementing a specific and costly experimental  
650 device.

#### 651 **4. Conclusion**

652 In this study, we investigated the added value of using high spectral res-  
653 olution to describe genotype responses to water stress using the REP-ASCA  
654 method. Two experimental campaigns were conducted in 2017 and 2018

655 where spectra were acquired from a panel of genotypes with different sen-  
656 sitivities to water stress. The 2018 season having a significant difference  
657 between the two irrigation conditions was used as a reference study of water  
658 stress situation. The spectra acquired during this campaign were analyzed  
659 by REP-ASCA. This method reduces errors due to lack of repeatability and  
660 provides scores and loadings for each of the identified terms.

661 We demonstrate that the treatment term loadings highlight the spectral  
662 regions impacted by the change in irrigation. When looking at yield potential  
663 regardless of treatment, genotypes can be grouped according to the loadings  
664 provided by the genotype term. And finally, we found out that the interaction  
665 term provides loadings used as new phenotyping traits describing genotype  
666 response to stress.

667 By projecting the observed spectra on these new components, we obtain  
668 scores that are useful to describe genotypes. The scores obtained for the  
669 interaction term were directly related to the percentage of the yield loss  
670 between the two irrigation conditions in a stressed environment. By applying  
671 these same components to spectra acquired on the same genotypes but in  
672 an environment with moderate water stress, we find the same classification  
673 for the majority of genotypes. This component seems to be best expressed  
674 when water reserves are limited, but is still relevant in the characterization  
675 of genotypes when the stress is light. This shows that REP-ASCA provides  
676 robust components applicable to environments with occasional stresses that  
677 do not impact yield. The analysis of scores simultaneously with loadings  
678 highlights the different strategies used by genotypes to manage water stress.  
679 Genotypes without adaptive mechanisms can suffer serious damage in terms

680 of growth, development and thus yield.

681 This study shows that the use of high-spectral resolution data, when  
682 linked to a method reducing repeatability error, is interesting to classify the  
683 behavior of maize genotypes to water stress.

684 Using NIR spectroscopy could be a preliminary study. A variable se-  
685 lection step on the spectral signatures obtained with REP-ASCA could be  
686 performed. New spectral indices could be created from a targeted breeding  
687 objective. While increasing the number of spectral bands, we need to be  
688 cautious about the possible redundancy of the spectral information. The use  
689 of high spectral resolution may be less useful in other applications, where  
690 spectral indices based on low resolution have proven to be very effective.  
691 But, provided that the right precautions are taken when processing the data,  
692 as here with REP-ASCA, high spectral resolution will be able to do as well  
693 as spectral indices, since the latter can be retrieved from high resolution  
694 information.

695 However, it would be interesting to build up a larger spectral database  
696 containing more genotypes and several drought typology environments. In  
697 addition, increasing the number of measurement dates for an experimental  
698 campaign would make it possible to monitor genotypes by focusing on their  
699 resilience to occasional water stress.

700 This study focused on identifying responses of different maize genotypes  
701 to water stress. The approach combining the REP-ASCA method with spec-  
702 tral data is adapted to other breeding objectives (diseases, hot stress or  
703 nitrogen deficiency) or even other crops.

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