

## Article

# Evaluation of Leaf Rust Resistance in the Spanish Core Collection of Tetraploid Wheat Landraces and Association with Ecogeographical Variables

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**Abstract:** Spain has a great landrace diversity of the subspecies of the tetraploid species *Triticum turgidum* L., namely, *durum* (or durum wheat), *turgidum* (or rivet wheat) and *dicoccon* (or domesticated emmer wheat). These wheats have to confront several foliar diseases such as the leaf rust. In this work, a core collection of 94 landraces of tetraploid wheats were inoculated with three leaf rust isolates. Besides, a larger collection (of 192 accessions) was evaluated in the field. Although the majority of landraces were susceptible, approximately 20% were resistant, especially domesticated emmer wheat landraces. Several variables, such as late heading and red coat seeds were associated to resistant accessions. Regarding ecogeographic variables, a higher rainfall from October to February and more uniform temperature were found in the area of origin of resistant landraces. Based on these results, several resistant landraces were identified that potentially may be used in durum wheat breeding programs. In addition, a predictive model was elaborated to develop smaller subsets for future screening with a higher hit rate for rust resistance.

**Keywords:** *Puccinia triticina*; *Triticum turgidum*; resistance; germplasm; ecogeographical variation



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## 1. Introduction

Several tetraploid wheats ( $2n = 4x = 28$ ) of the species *Triticum turgidum* L. are cultivated. Among them, durum wheat (*T. turgidum* subsp. *durum* (Desf.) Husn.) is the most important crop with a yearly acreage of about 16 million ha and a production of 37 million t [1]. Domesticated emmer wheat (*T. turgidum* subsp. *dicoccon* (Schrank) Thell.) and rivet or poulard wheat (*T. turgidum* L. subsp. *turgidum*) are also cultivated crops. Domesticated emmer wheat is a hulled grain wheat that was very important in the past, but today remains as a relict crop in isolated areas of Italy, Ethiopia, Iran, and India [2]. Rivet wheat, also a minor crop, is similar to durum wheat but the spike (lax and long, with rough awns that can fall off at maturity) and the kernel (naked when threshed, but round and soft) are different.

Rusts are important diseases in wheat since they are dynamic pathogens and affect wheat worldwide [3]. Leaf (or brown) rust is a foliar disease caused by the fungus *Puccinia triticina* Eriks. It is the most constant disease globally out of the three rusts diseases (yellow rust, leaf rust, and stem rust). It affects to bread wheat, durum wheat, and triticale. Although durum wheat was generally deemed more resistant to leaf rust compared to bread wheat, from 1998 to 2006 severe leaf rust outbreaks on durum wheat were recorded at many locations in southern Spain. Most durum wheat cultivars rendered susceptible

at that time in Spain, with a few exceptions such as the Italian cultivar Colosseo [4]. That moment almost coincided with severe outbreaks in Mexico and many other parts of the world [5]. Since then, many resistant cultivars were released mostly from CIMMYT or from Italian breeding companies. Most resistant cultivars carried *Lr14a* gene or the closely linked *QLr.ubo-7B.2* (present in Colosseo) located on chromosome 7BL [6]. Other resistant cultivars carry the complementary genes *Lr27 + Lr31* (that originated from bread wheat but transferred to several durum wheat cultivars such as Jupare C2001, and LrCamayo (present in cultivar Camayo), but nonetheless, the resistant stock is scarce.

Two features are typical of the leaf rust of durum wheat compared to bread wheat leaf rust isolates. First, races that affect most of the world (except Ethiopia) have a similar virulence profile and, presumably, a common origin [7]. However, this fact does not prevent new virulent races from occurring by mutation and selection against the main *Lr* genes deployed in durum wheat [8]. Second, it is the existence of a sibling species called *Puccinia tritici-duri* V.-Bourgin, that was described first in Morocco, and have *Anchusa azurea* Mill. as alternate host. This species displays larger pustules than common leaf rust, with a rapid tendency to form telia on the same position of the pustule [9]. Most *Lr* genes present in durum cultivars does not activate the resistance when the plant is challenged by this leaf rust species.

Apart from the normal hypersensitive resistance based on single, major, and race specific *Lr* genes, another type of resistance called partial resistance (PR) has contrasting features. It is quantitative, race nonspecific (horizontal), and based on minor genes, although some of those genes show greater effect than others. The resistance is normally durable, i.e., it is effective for a long period of time in a prone environment to disease, in contrast with the hypersensitive resistance, with may be overcome when a new race develops a mutation in the avirulence loci [10]. Partial resistance to wheat leaf rust was first described in bread wheat [11,12], where several cultivars showed to possess fair levels of PR (Frontana, Parula, Pavon 76, etc.). Some genes for partial resistance have been characterized, as *Lr34* and *Lr46*, and, interestingly, these genes have a pleiotropic effect, that is, they provided resistance to other diseases [13]. Although PR to rust was first defined in field experiments, displaying a typical “slow rusting” in the progress of the disease, the components of this resistance can be measured in greenhouse or growth chamber in monocyclic experiments. Therefore, parameters like the latency period (time between inoculation and the time at which 50% of the final number of pustules appeared) [14] or the uredinium size are well correlated with the slow rusting in the field. In durum wheat, works on PR to leaf rust in several cultivars (such as Planeta) demonstrated that uredinium size was the best predictor of partial resistance, followed by the latency period [15,16].

Landraces are characterized by a specific adaptation to the environmental conditions of the area of cultivation [17] where the selection of resistant genotypes in some populations is an adaptive response to the biotic stress caused by the pathogen. There are some examples of landraces as source of resistant genes to rust: the Levantine landrace Gaza carries *Lr23* [18], while the Portuguese accessions AUS 26582 and AUS 26579 carries the *Lr61* present in the cultivar Guayacán [19]. In durum wheat landraces coming from the Iberian Peninsula has been also found leaf rust resistance [20]. Additionally, landraces may have fair levels of partial resistance. Farmers (aided by natural selection) have made a selection against extreme rust susceptibility across seasons in rust prone areas [21,22].

The evaluation of landraces collections for rust response is a useful approach for exploring novel variation and determine their potential as sources for favourable alleles conferring resistance. However, the number of relevant accessions in genebank collections available to be evaluated is often substantially larger than the capacity of the evaluation project. An efficient strategy to mining genetic resource collections is to carefully screen core collections where the genetic diversity is maximized, and the number of accessions is lower than is normally required for evaluation to identify novel variation. A core collection of 94 genotypes comprising landraces of the three tetraploid subspecies is currently preserved in the CRF-INIA (Centro Nacional de Recursos Fitogenéticos, National Plant Genetic

Resources Centre) at Alcalá de Henares (Madrid) [23]. Several studies have demonstrated the high genetic variability of these collections for different traits relevant for wheat improvement [24–26].

It has been reported that wheat landraces with the highest level of resistance to some diseases originated from sites where diseases pressure was high, due to environmental factors [21,27]. Therefore, the ecogeographic characterization of the landraces cultivation sites can be very useful in explaining the suitable eco-geography where the pathogen thrives and thus are likely to impose a selection pressure for the emergence of resistance genes. The relation between resistance expression in the evaluated accessions and the ecogeographic parameters of the collection site allow to detect novel variation in other populations originating from locations with an environmental profile similar to the collection sites of the reference set of accessions with known resistance. Different studies analysing the associations between ecogeographic variables and rust resistance in common wheat demonstrated a strong environmental component in the geographic distribution of rust resistance genes [28–31]. Nevertheless, few studies have focussed on leaf rust of durum wheat.

The objective of this work was to evaluate the resistance to several isolates of leaf rust in the Spanish core collection of tetraploid wheat and to relate the origin of the resistant landraces with local ecogeographical variables.

## 2. Materials and Methods

### 2.1. Plant Material

The Spanish core collection of tetraploid wheat used in the analysis of rust resistance at seedling stage in greenhouse comprised 94 landraces of three subspecies of the species *Triticum turgidum* L. (10 of domesticated emmer wheat, 32 of rivet wheat, and 52 of durum wheat). Four essays were carried out in seedlings, three to evaluate the hypersensitive resistance to leaf rust with three different isolates, and the other to characterize the partial resistance of the collection to one of the isolates. The cultivar Don Rafael was used as susceptible check, while cultivar Don Valentín (*Lr27 + Lr31*) was utilized as resistant check, and cultivar Planeta as partially resistant check.

Another set of 192 Spanish landraces of tetraploid wheat (14 of domesticated emmer wheat, 38 of rivet wheat, and 140 of durum wheat), which included the core collection, was evaluated at adult plant stage in field experiments. This set was representative of the entire collection of 552 accessions maintained at the CRF and was used as primary subset to select the core collection [32].

### 2.2. Fungal Material

The isolates of *Puccinia triticina* used in this study were Conil Don Jaime 13 (CDJ13) and Jerez Don José 15 (JDJ15), both collected from durum wheat fields in southern Spain, and Peralta García 14 (PG14), collected from common wheat in northern Spain. Inoculation on a Thatcher/*Lr* isolines differential set showed that the isolates were virulence/avirulence on the following *Lr* genes:

- CDJ13: *Lr10, Lr14a, Lr14b, Lr20, LrB / Lr1, Lr2a, Lr2c, Lr3, Lr3bg, Lr3ka, Lr9, Lr11, Lr16, Lr17, Lr18, Lr24, Lr26, Lr28, Lr30.*
- JDJ15: *Lr1, Lr10, Lr14b, Lr18, Lr20, LrB / Lr2a, Lr2c, Lr3, Lr3bg, Lr3ka, Lr9, Lr11, Lr14a, Lr16, Lr17, Lr18, Lr24, Lr26, Lr28, Lr30.*
- PG14: *Lr1, Lr3, Lr3bg, Lr10, Lr14a, Lr14b, Lr16, Lr17, Lr18, Lr20, Lr26, Lr30, LrB / Lr2a, Lr2c, Lr3ka, Lr9, Lr11, Lr18, Lr24, Lr28.*

### 2.3. Experiment of Hypersensitive Resistance at Seedling Stage in the Greenhouse

Landraces of the core collection were sown in plastic trays of 60 × 40 cm in soil made of peat moss and sand (1:1 v./v.). In each tray, four plants of 16 landraces plus the susceptible and resistant check were grown in a greenhouse at Technical School of Agricultural Engineering (ETSIA, University of Seville, Seville, Spain). Inoculations were

performed in two different experiments at two different plant seedling stages, first and fifth leaf (12 and 16 Zadoks scale) [33]. Plants were inoculated by dusting 4 mg of uredospores per tray mixed with talc powder (1:40 v./v.), which resulted in a deposition of about 70 spores/cm<sup>2</sup>. Leaves were laid and fixed on the soil with metallic hairpins. Inoculated plants were placed in an incubation compartment within the greenhouse at 18–20 °C, with darkness and humidity at saturation for 15 h. The next day, hairpins were removed from the leaves and plants were transferred back to their greenhouse compartment. At 13 days after inoculation, when the number of pustules in the susceptible check no longer increased, infection type was assessed in each leaf of the landraces, using the McNeal scale [34]. In most cases, infection type score agreed in all four leaves of each landrace. This scale is a 0–9, where infection type lower than 7 indicated a resistant or incompatible response in the landraces, while an infection type of 7 or more indicated a susceptible or compatible response.

#### 2.4. Experiment of Partial Resistance at Seedling Stage in the Greenhouse

For this experiment, the 75 landraces that showed a high infection type ( $IT \geq 7$ ) when inoculated with isolate CDJ13 were sown again in plastic trays, but the resistant check was replaced by the partially resistant cultivar Planeta. Four first leaves of each landrace were inoculated this time using a settling tower to improve the uniformity of the inoculation. The length (L) and the width (W) of eight uredinia per leaf and genotype were measured with a binocular microscope. From these measures the surface of the uredinium, considered as an ellipse, was  $\pi/4 \times L \times W$ . This trait is highly correlated with partial resistance to leaf rust of durum wheat [15].

#### 2.5. Leaf Rust Evaluation at Adult Plant Stage in the Field Experiments

Wheat landraces were tested for leaf rust in adult plant in field plots in 2007–2008 at Jerez de la Frontera (36°43'42" N, 6°09'46" W), a southern Spanish region prone to wheat rust disease [4]. The accessions were sown in an augmented design [35] in plots of 3 rows of 2.5 m length and 15 cm row spacing. Two durum wheat cultivars widely cultivated in the country, Simeto and Vitrón, were included in each block as checks. Both cultivars are susceptible to the leaf rust races developed after 1998. Field infection was natural and leaf rust severity was assessed following the modified Cobb scale [36], expressed as a proportion of foliar surface covered by pustules with respect to the total plant (from 0 to 100%). Accessions with leaf rust severity from 0 to 10% were considered resistant, since hypersensitive resistance usually results in fewer and smaller pustules that reduce greatly the severity [3], and those with severity higher than 10% were regarded as susceptible.

#### 2.6. Agronomic Characterisation

Four qualitative agromorphological traits (growth habit, spike density, glume hairiness, and seed colour) were obtained from a previous study [32] carried out during the season 2006–2007 at the centre of Spain (Alcala de Henares, Madrid). Three quantitative agromorphological traits (days to heading and to maturity, and plant height) were obtained from that study and also recorded from the rust resistance field experiment in 2007–2008. All traits were evaluated according to the International Board of Plant Genetic Resources (IBPGR) from five different plants in each accession (Table S1).

#### 2.7. Ecogeographic Characterisation

Ninety-one accessions of the core collection and 177 accessions of the whole set evaluated in field plots were assigned to one of the nine agroecological zones for durum wheat defined on the basis of historical yield records and province of origin of the landrace [32]. Geographic coordinates (latitude, longitude and altitude) of the collection site of each landrace were obtained for 54 accessions of the core collection and 84 accessions evaluated in the field trial from the passport data of each accession ([http://webx.inia.es/web\\_inventario\\_nacional/Introduccioneng.asp](http://webx.inia.es/web_inventario_nacional/Introduccioneng.asp), accessed on 23 March 2021). The accuracy of the geographic

coordinates was checked with Google Earth ([www.google.com/earth/index.html](http://www.google.com/earth/index.html), accessed on 23 March 2021). Data on 75 ecogeographic variables classified into three ecogeographical components were gathered: bioclimatic variables (67), geophysic variables (3) and edaphic variables (5) (see Table S2). The bioclimatic variables (1950–2000 period) were related to temperature and rainfall, including some indexes which analysed the relationships between both climatic effects. The geophysic variables were related to solar radiation (northness, eastness, and elevation), and the edaphic parameters were related to the physical and/or chemical conditions of the soil (pH, bulk density, and clay and sand content). The values of the ecogeographic variables were extracted for each collection site from raster layers with a 2.5 arc min resolution ( $5 \times 5$  km cell grid) obtained from various sources compiled by CAPFITOGEN v. 2.0 [37].

### 2.8. Data Analyses

For qualitative traits, significant differences between the frequencies in the rust resistant and susceptible groups were checked by chi-squared test ( $p$ -value  $< 0.05$ ). For quantitative variables, a homogeneity test (Levene's test) for variances and a t-test for means ( $p$ -value  $< 0.05$ ) were used to compare the resistant and susceptible groups. For those variables deviated from equality of variances, the nonparametric Kruskal and Wallis test was used [38]. Relationships between variables were examined using Pearson correlation coefficient ( $p < 0.05$ ).

For the core collection evaluated at the seedling stage, a predictive model was elaborated for each isolate with the random forest (RF) clustering algorithm using the bioclimatic data (explanatory variables) and the leaf rust resistant/susceptible classes (dependent binary variable). This procedure differs from standard tree classifier in that it "grows" many classification trees in the process, leading to higher classification accuracy than other classifiers [39]. The number of variables in the random subset at each split node ( $m_{try}$ ) were optimized for each model by monitoring the magnitude of the mean square prediction error (rate of classification error) observed in the out-of-bag (OOB) set; that is, the ability of each iteration to correctly classify an unknown accession as resistant or susceptible. The number of trees in the forest ( $n_{tree}$ ) was 1000 for all the models.

Two "test" sets of accessions screened for adult plant resistance in field plots were used to evaluate the prediction performance of the model obtained with the RF approach: one including all the accessions (set 1), and other with accessions not included to build the model (set 2). The model was supplied with the bioclimatic data of the two sets of accessions and the results of the predictions were validated by the disease evaluation scores recorded in the field tests. All the accessions of set 2 were susceptible at the adult stage in the field trial, so a third set (set 3) which included five accessions from the core collection, resistant under field conditions, was also used to evaluate the model. No model was constructed for the common wheat isolate since this model was not possible to be evaluated with data from field experiments.

The agreement between the models' predictions and the disease scores in the field plots were quantified with the Kappa parameters: sensitivity, specificity, Kappa coefficient, and accuracy. These parameters were derived from the  $2 \times 2$  contingency table (Table 1). Sensitivity, defined by  $a/(a + c)$ , and specificity, defined by  $d/(b + d)$ , are indicators of the model's ability to correctly classify observations as either resistant or susceptible. The higher the values of sensitivity and specificity, the lower the error and thus the better the discriminating power of the model. The Kappa coefficient was used to assess improvement over chance and measures the specific agreement in the confusion matrix table. Thus, a high value is an indication that the models performance were adequate for prediction purposes [40]. A value of Kappa below 0.4 was an indication of poor agreement and a value of 0.4 and above was an indication of good agreement [41]. The overall correct classification was defined by  $(a + d)/n$ .

**Table 1.** Confusion matrix (2 × 2 contingency table).

		Observed	
		Resistant	Susceptible
Predicted	Resistant	a	b
	Susceptible	c	d

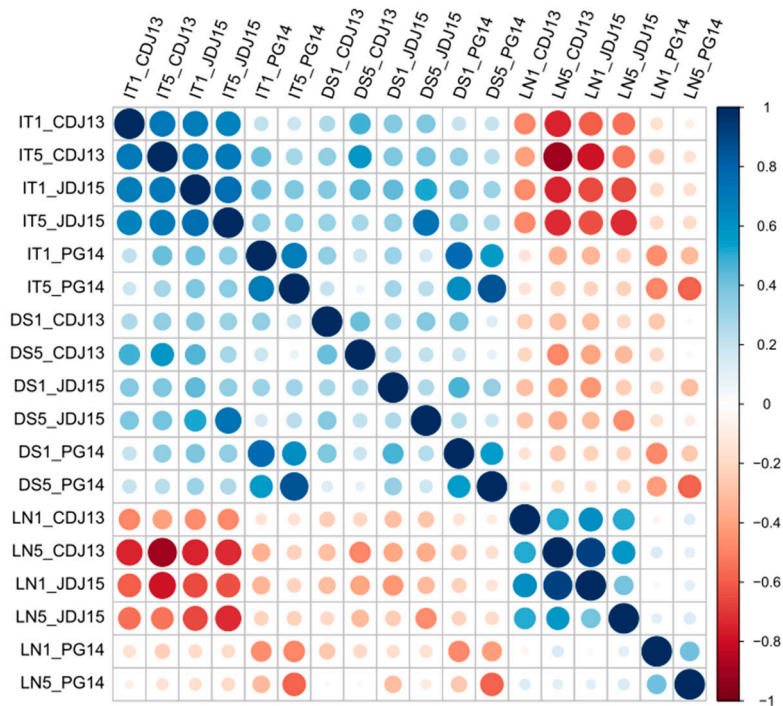
The RF analysis provides a ranking of the most important variables influencing the resistant/susceptible classification, placing variables with a higher mean decrease in accuracy in the first positions. The variables in the top 30 variables of the ranking with Pearson correlation coefficient  $r > |0.50|$  and  $p < 0.05$  were considered correlated variables. Statistical analyses were performed with the software R version 3.6.3 and Infostat version 6.12 software.

**3. Results**

**3.1. Leaf Rust Resistance of the Core Collection at Seedling Stage in the Greenhouse**

**3.1.1. Correlations between Disease Parameters**

The Spanish core collection of 94 accessions of the three *T. turgidum* subspecies, domesticated emmer wheat (10 accessions), rivet wheat (32 accessions), and durum wheat (52 accessions) were evaluated for leaf rust resistance to the rust isolates CDJ13, JDJ15 and PG14 at first and fifth leaf stages in greenhouse tests. Three parameters were tested: the infection type (IT), disease severity (DS) and leaf necrosis (LN) (Table S3). Correlation analyses detected significant correlations between the first and fifth leaf scores for the three parameters within each isolate (Figure 1). DS was positively and negatively correlated with IT and LN, respectively, in all the isolates. Between isolates, the highest correlations were between CDJ13 and JDJ15 for the three parameters assessed.



**Figure 1.** Pearson correlations between the disease parameters infection type (IT), disease severity (DS) and leaf necrosis (LN) assessed for each isolate Conil Don Jaime 13 (CDJ13), Jerez Don José 15 (JDJ15) and Peralta García 14 (PG14). The number 1 and 5 after the disease parameter indicated first and fifth leaf, respectively. Coefficients  $> |0.2|$  are significant ( $p < 0.05$ ).

### 3.1.2. Identification of Resistant Accessions to Leaf Rust

Resistant accessions to each isolate were identified by their IT of leaf rust at the fifth leaf stage. Although first and fifth leaf scores were related, the fifth leaf response was better correlated with the adult plant response since several adult plant genes are expressed since the fifth leaf rust stage [42]. Thus, those accessions with IT values at fifth leaf lower than 7 were considered resistant to the corresponding isolate. The number of susceptible accessions to the leaf rust isolates CDJ13 and JDJ15 was higher than the number of resistant accessions, whereas the latter was predominant for PG14 (Table 2).

**Table 2.** Number of resistant accessions (IT < 7) to specific isolates of leaf rust at the seedling stage in the core collection of tetraploid wheat.

Subspecies/Isolate	No.	CDJ13		JDJ15		PG14	
		No.	%	No.	%	No.	%
Domesticated emmer wheat	10	6	67	6	75	10	100
Rivet wheat	32	7	23	6	20	18	56
Durum wheat	52	7	14	12	24	47	90
Total	94	20	22	24	28	75	80

CDJ13 = Conil Don Jaime 13, JDJ15 = Jerez Don José 15, PG14 = Peralta García 14.

Seven durum landraces, six rivet and five domesticated emmer wheat accessions of the core collection presented simultaneous resistance against the three leaf rust isolates. The subspecies showed significant differences in the number of resistant accessions within each isolate ( $p = 0.0013$ ,  $0.02$  and  $0.0008$  for CDJ13, JDJ15 and PG14, respectively). A higher frequency of resistant accessions to CDJ13 and JDJ15 occurred among the accessions of domesticated emmer wheat than among durum and rivet wheat accessions (Table 2). For PG14, both domesticated emmer and durum wheat included a higher frequency of resistant accessions than rivet wheat. Furthermore, all the analysed domesticated emmer wheat accessions were resistant to PG14.

### 3.1.3. Relations between Seedling Resistance and Agronomic Traits

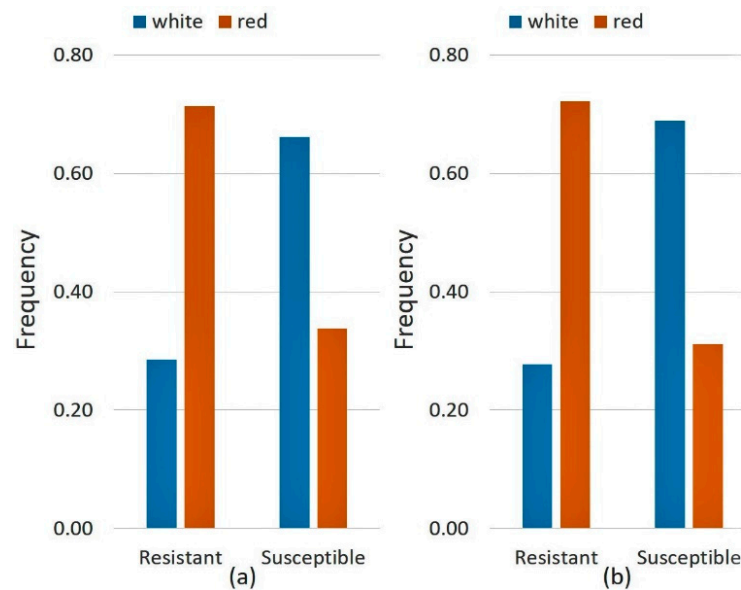
The relationship between agromorphological traits and disease resistance was evaluated for durum and rivet wheat. Domesticated emmer wheat was not included in the analyses because this subspecies has a typical agrotypic very different from those of the other two subspecies. For the qualitative morphological traits (Table S1), resistant accessions to CDJ13 and JDJ15 showed a higher frequency of red seeds than white seeds, while an opposite tendency was detected for the susceptible group ( $p < 0.01$ ) (Figure 2). None of the morphological characters growth habit, spike density and glume hairiness showed significant associations with leaf rust resistance to any of the three isolates.

For the quantitative agronomic traits, days to heading and maturity, and plant height (Table S1), the only significant difference detected was that resistant accessions to CDJ13 had a higher number of days to heading than those recorded as susceptible (176 days vs. 172 days,  $p = 0.0075$ ).

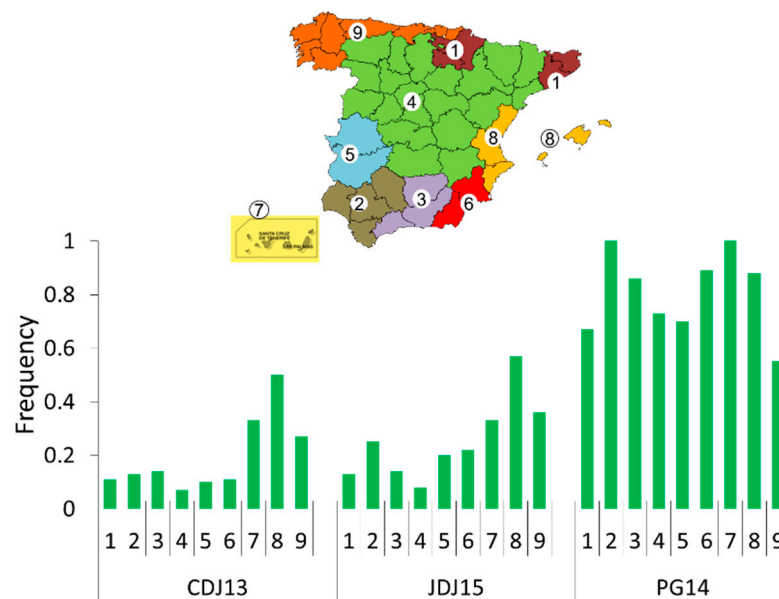
### 3.1.4. Relations between Seedling Resistance and Ecogeographic Variables of the Collection Site

The association of evaluation data for leaf rust resistance with the environmental conditions of the collection sites of the assessed accessions can help to identify environments that are likely to impose selection pressure for the emergence of resistance genes. In the present study, domesticated emmer wheat was not included in the analyses to avoid possible deviations, since this subspecies is traditionally cultivated in a unique ecogeographic region in the north of the country, where durum wheat is not usually cultivated. In contrast, durum and rivet wheat growth sites are more widely distributed across the country. Most of the sites of the durum and rivet wheat accessions were ascribed to one of the nine agroecological zones in Spain [32]. Although some zones were represented by a

low number of accessions in the present study, for CDJ13 and JDJ15 the highest frequency of resistant accessions was found in the eastern zone of Spain (Zone 8) (Figure 3).



**Figure 2.** Seed color frequency in resistant and susceptible accessions to (a) Conil Don Jaime 13 and (b) Jerez Don José 15 isolates at seedling stage for durum and rivet wheat of the core collection.



**Figure 3.** Frequency of resistant accessions at seedling stage in each of the nine agroecological zones of Spain defined by [32] in durum and rivet wheat within each isolate Conil Don Jaime 13 (CDJ13), Jerez Don José 15 (JDJ15) and Peralta García 14 (PG14).

Relationships between disease resistance and 75 ecogeographic variables of the collection site in Spain were analysed for durum and rivet wheat (Table S2). Resistant and susceptible accessions showed significant differences for the ecogeographic variables related to their collection site for the isolates CDJ13 and JDJ15, but none was significant for the geophysic or edaphic variables (Table 3 and Table S4). The highest number of differences between both resistant and susceptible groups was detected for JDJ15. For that isolate, both bioclimatic thermal and hydric variables showed contrasting differences. In general,



resistant accessions came from areas with more uniform temperatures, lower maximum temperatures in the hottest period, and higher precipitation in the coldest season. Resistant accessions for CDJ13 usually came from zones with higher isothermality.

**Table 3.** Ecogeographic variables ordered according to their significant differences ( $p < 0.05$ ) between resistant and susceptible accessions at seedling stage for the durum and rivet wheat of the core collection.

Isolate	Bioclimatic (Thermal Variables)	Bioclimatic (Hydric Variables)
CDJ13	Isothermality (+) <sup>1</sup>	-
JDJ15	Temperature Seasonality (–) <u>Annual temperature range</u> (–) <u>Mean daily temperature range</u> (–) July maximum temperature (–) Maximum temperature of hottest month (–) August maximum temperature (–) July mean temperature (–)	<u>October precipitation</u> (+) <u>November precipitation</u> (+) <u>Precipitation of wettest month</u> (+) <u>Precipitation of wettest quarter</u> (+) December precipitation (+) Annual precipitation (+) January precipitation (+) <u>Precipitation of coldest quarter</u> (+) February precipitation (+)

<sup>1</sup> The (+) or (–) indicates the associations of high or low values of the variable with the resistant accessions. The variables that were also significant different between resistant and susceptible accessions in adult plants in the field are underlined. CDJ13 = Conil Don Jaime 13, JDJ15 = Jerez Don José 15.

The classification model obtained with the Random Forest approach for both durum wheat isolates indicated that thermal variables were more relevant in the model than hydric variables (Table S5). The identification of the most significant no correlated variables confirmed the influence of the temperature uniformity for both isolates, and autumn precipitation for JDJ15 (Table 4).

**Table 4.** The most significant bioclimatic variables not correlated in the top 30 variables selected according to the mean decrease accuracy of the random forest approach for durum and rivet wheat within each isolate at seedling stage.

Isolate	Bioclimatic (Thermal Variables)	Bioclimatic (Hydric Variables)
CDJ13	Annual temperature range April maximum temperature Isothermality	
JDJ15	February maximum temperature Annual temperature range Isothermality	November precipitation

CDJ13 = Conil Don Jaime 13, JDJ15 = Jerez Don José 15.

### 3.2. Leaf rust Resistance at Adult Plant Stage in the Field Experiments

A total of 192 tetraploid landraces (including the core collection) were tested for leaf rust severity in field plots in the south of Spain (Table S6). The two check cultivars, Simeto and Vitrón, showed a high susceptibility, with a leaf rust severity of 90%. Significant correlations were obtained between disease severity in the field (adult plant stage) and the IT values assessed for the landraces at the seedling stage for CDJ13 ( $r = 0.55$ ,  $p < 0.0001$ ) and JDJ15 ( $r = 0.60$ ,  $p < 0.0001$ ). A lower correlation was obtained for the common wheat isolate PG14 ( $r = 0.19$ ,  $p = 0.05$ ) (Figure S1).

The three subspecies showed no significant differences in the number of resistant accessions (severity values lower than 10%) at the adult plant stage. However, significant differences for the severity level, which measured the foliar surface percentage covered by uredinia, existed among the subspecies ( $p < 0.0001$ ); domesticated emmer wheat having the

lowest values (40.7%), rivet wheat occupied an intermediate position (58.2%), and durum having the highest values (67.8%).

Similarly, to the results at seedling stage, resistant accessions of durum and rivet wheat had a higher frequency of red seeds (63%) than those being susceptible (26%) ( $p = 0.02$ ). For these two subspecies, no significant differences were found among the agroecological zones, probably due to the low number of resistant accessions, but the associations with the ecogeographic variables indicated that accessions from areas with more stable and uniform temperatures, and more precipitation in October showed generally low severity values (Table 3 and Table S7).

The classification models obtained for the two durum wheat isolates with the seedling tests were used to predict which accessions might be resistant or susceptible at the adult plant stage. All the accuracy parameters indicated a good agreement between predictions and score data in the field plots for the three sets of accessions evaluated (Table 5). Some coefficients could not be calculated for set 2, which included only accessions not used to build the model, because all the accessions were susceptible at the adult stage. Both models had the same sensitivity for sets 1 and 3 (both identified 4 of the 5 resistant accessions in the field trial), although the model obtained for CDJ13 showed a better predictive power when all the accessions were used (set 1).

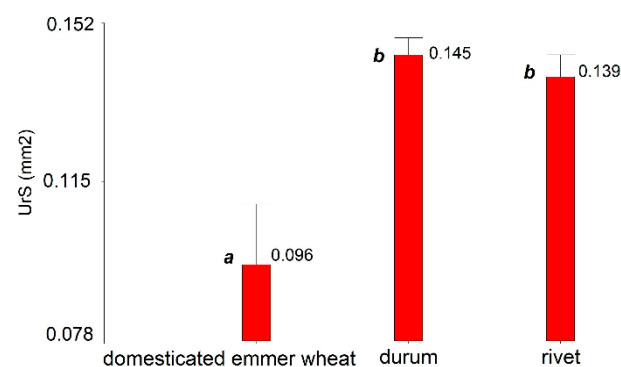
**Table 5.** Accuracy of the random forest classification of different sets of accessions assessed at adult plant stage in the field experiments.

		Correct Classification (Frequency)	Kappa Coefficient	Sensitivity (Frequency)	Specificity (Frequency)
CDJ13	set 1 <sup>1</sup>	0.93	0.58	0.80	0.94
	set 2	1.00	na	na	1.00
	set 3	0.97	0.87	0.80	1.00
JDJ15	set 1	0.89	0.45	0.80	0.90
	set 2	1.00	na	na	1.00
	set 3	0.97	0.87	0.80	1.00

<sup>1</sup> set 1 = all the evaluated accessions, set 2 = accessions not used to build the model, set 3 = accessions of set 2 plus five accessions of the core collection. CDJ13 = Conil Don Jaime 13, JDJ15 = Jerez Don José 15.

### 3.3. Partial Resistance to Leaf Rust at Seedling Stage in the Greenhouse

Partial resistance to CDJ13 was assessed in 74 susceptible accessions ( $IT \geq 7$ ) by measuring the uredinium size (Table S8). One accession of domesticated emmer wheat (BGE048901) showed a significant lower uredinium size ( $UrS = 0.059 \text{ mm}^2$ ) than the partially resistant check “Planeta” ( $UrS = 0.087 \text{ mm}^2$ ). Domesticated emmer wheat also displayed a lower uredinium size than rivet and durum wheat (Figure 4), indicating that domesticated emmer wheat accessions manifested a higher level of partial resistance.



**Figure 4.** Mean values of uredinium size ( $UrS$ ) of leaf rust at seedling stage for the three subspecies of *T. turgidum*. Means with different letters are significantly different at  $p = 0.05$ .

Correlations between agromorphological traits and uredinium size were not significant, except for days to heading which showed a negative correlation with UrS ( $p = 0.02$ ). Correlation analyses performed separately for each subspecies detected that UrS was negatively correlated with days to maturity ( $p = 0.05$ ) and positively correlated with growth habit ( $p = 0.02$ ) only in rivet wheat.

Separate analyses for each subspecies revealed no significant correlations between UrS and the ecogeographic characteristics of the collection site. In contrast, the combined analyses for the three subspecies indicated that partial resistance increased significantly ( $p < 0.05$ ) with a decrease in the temperature of the landrace site of origin, mainly in spring (UrS positively correlated with annual temperature, April to June and September mean temperature, April and May maximum temperature, March to June and September to November minimum temperature (Table S9).

#### 4. Discussion

The Spanish core collection of tetraploid wheat (*Triticum turgidum* L.) assessed in this research represents the genetic variability of more than 550 traditional varieties coming from all the agroecological zones of durum wheat cultivation in the country. Therefore, the variability found for leaf rust resistance may reflect, to a certain extent, the adaptive value of such genetic diversity to the environmental conditions prevailing in each region. Previous studies have shown that this collection possesses a high genetic variability for important adaptive traits related to the origin site [24,32].

Some of the Spanish landraces showed hypersensitive resistance to the three isolates evaluated at seedling stage. Different resistance expressions to the three isolates were detected. Eighteen accessions expressed resistance to the three isolates simultaneously (63%, 20% and 14% of the domesticated emmer, rivet and durum wheat accessions, respectively). All the susceptible accessions to the common wheat leaf rust isolate (PG14) were also susceptible to both durum wheat leaf rust isolates (especially in rivet wheat), whereas the accessions resistant to one of the durum wheat isolates (CDJ13 or JDJ15) were also resistant to the common wheat isolate. Other accessions, despite showing resistance to the common wheat leaf rust isolate, were susceptible to the two durum wheat leaf rust isolates. These results seemed to indicate that resistance genes to one of the durum wheat isolates provided resistance to the common wheat isolate, but some accessions (mostly of subsp. *durum*) could have other different resistance genes that are effective to common wheat leaf rust isolate. These different reactions to common and durum wheat isolates agreed with the results reported by [5]. These authors found an average similarity in virulence of 60% between different common wheat leaf rust isolates and a worldwide collection of leaf rust from durum wheat.

The studied accessions displayed higher resistance to the common wheat isolate than to the durum wheat isolates at the seedling stage in agreement with the finding in a durum wheat worldwide collection [43]. The common wheat isolate analysed PG14 is one of the races that inflict yield losses in common wheat crop in northern Spain. Our results confirm that common wheat isolates are not adapted to infect durum wheat, and therefore most durum genotypes are resistant to isolates from common wheat. According to other studies, many durum lines and cultivars possess *Lr72* that protect them against most of common wheat isolates [5]. In fact, *Lr72* gene was present in 85% of the CIMMYT durum wheat lines by 2001 [18]. However, in the case of landraces it seems that this gene is less frequent or absent, so other genes, especially present in the domesticated emmer and durum wheats could confer cross resistance [5].

The two durum wheat isolates developed in Spain in different time periods. The CDJ13 represents the new races detected in 2013, virulent for the main leaf rust genes *Lr14a* and *Lr72* [44]. In contrast, JDJ15, collected in one of the most important endemic zones of rust in Spain, although collected in 2015, represented the older races, avirulent to *Lr14a* gene. A strong relationship between the two durum wheat leaf rust isolates was detected based on the significant correlations obtained between them for the three infection

parameters (infection type, disease severity and leaf necrosis). Accordingly, the 90% and 75% of the accessions expressing resistance to CDJ13 and JDJ15, respectively, were resistant to both isolates, which is in agreement with durum wheat isolates from Europe had an average similarity in virulence of 90% [5,45]. This result indicated that these resistant genotypes have *R*-genes that are effective to both durum wheat isolates. That gene might be one of the few known genes present in durum wheat (i.e., *Lr23*, *Lr61*, *LrCam*) [46] or can be a new gene, not described so far. These genotypes were more frequent in accessions of domesticated emmer wheat and less common in those of durum. Seven accessions (one domesticated emmer and six durum wheat) showed resistance to JDJ15, but not to CDJ13, which could be due to the presence of an allele of the gen *Lr14* [47]. Only one accession (of rivet wheat) was resistant to CDJ13 and susceptible to JDJ15. Despite the new races that appeared in 2013 were more virulent, it seems that sources of resistance effective to those races can be found in the Spanish landraces.

The rust resistance values assessed for the two isolates from durum wheat at the seedling stage were significantly related to those obtained in adult plants in field plots, suggesting that these two isolates were similar to those present in the field. It is remarkable that field evaluation took place in Jerez de la Frontera, near the Lower Guadalquivir Valley, a Spanish region prone to wheat rust disease [4]. The higher correlation of the field data with the results of JDJ15 (collected at Jerez) respect to those with CDJ13 was in agreement with this latter developed in Spain in the following years (2013), and probably was absent in the field when the evaluation was carried out in 2008. At this time, races more similar to JDJ15 were probably present in the field. The significant correlation, however, obtained between resistance values to CDJ13 and severity values in the field confirmed the high similarity between both durum wheat isolates. It thus seems reasonable to use the field results to confirm the associations found with seedling resistance.

The evaluation of partial rust resistance is of relevance because a key characteristic of genes expressing a partial resistance is that they confer resistance to all known races of *P. triticina* (horizontal resistance). However, these (minor) genes do not provide complete resistance that is manifested by many *R*-genes conferring hypersensitive resistance with no uredinia produced. Nonetheless, these genes provide durable resistance since virulent forms of *P. triticina* have not yet been detected [48,49]. In a study relating leaf rust resistance of European common wheat landraces and their origin, it was found that regions with high or intermediate severity of leaf rust led to landraces with a higher level of partial resistance. [21] In the present study, partial resistance to leaf rust was correlated with late heading and low temperatures of the origin site, mainly in spring. Although only three domesticated emmer wheat accessions were included in the analyses, their presence could influence the obtained results. However, it is remarkable that thermal variables were the only ecogeographic data correlated with the partial resistance, despite the significant differences observed in the hydric variables between the collection sites of domesticated emmer wheat and the other two subspecies in the accessions analysed. Separate analyses for each subspecies revealed that a higher resistance level was related with late heading and prostrate plant habit in rivet wheat. These agronomic characteristics are adaptive traits to cold areas. It thus seems that lateness and colder collection zones could be a criterion to select Spanish landraces of durum wheat with higher probability of possessing partial resistance to leaf rust. Partial resistance is a quantitative trait, largely affected by diverse factors of the environment. Low temperature increases the difference in leaf rust severity between a susceptible and a partially resistant genotype [50]. Thus, in a colder, although slightly, environment during the wheat growing season, it is more likely that the farmers of the past more easily identified a partially resistant landrace.

The analysis of each subspecies to the leaf rust response variability indicated that domesticated emmer wheat presented a greater resistance in all the evaluations (at seedling and adult plant stages, and for partial resistance). These results confirmed the interest of this subspecies as a donor of genes for resistance to pathogens [51,52]. In the case of leaf rust, domesticated emmer wheat is the origin of the *Lr14a* gene and of a novel adult-

plant resistance genes, *Lrac104* [53,54]. Spanish domesticated emmer landraces can be of particular interest as genetic analyses have indicated that they are quite distinct from other domesticated emmers [55]. In general, the subsp. *durum* had a better resistance response to the common wheat isolates than the rivet wheat, in spite of the high genetic similarity between both subspecies [32,55].

A clear association was detected between seed colour and seedling resistance or rust severity in adult plants. In both cases, the resistant genotypes had a higher frequency of red seeds. This relationship is consistent with the fact that domesticated emmer wheat had red seeds and was the most resistant subspecies to both isolates. These observations indicate a genetic linkage between resistance and seed colour genes in the landraces analysed. In a previous study, [56] a significant association between seed colour and some DArTs markers (rPt-0996 and wPt-0665) located on chromosome 3BL in a set of Spanish landraces was found. The former marker was also associated to endosperm yellow pigment. In the present study, a significant positive correlation between IT values for JDJ15 and yellow pigment (data from [55]) was detected for the subsp. *durum* ( $r = 0.54$ ,  $p = 0.0001$ ), which seems to confirm that some resistant genes could be located on chromosome 3BL. Different *Lr* genes have been described on this chromosome arm such as the newly described gene *Lr77*, derived from the hard red winter wheat cultivar Santa Fe [57], and the *Lr79* from the Portuguese durum wheat landrace AUS26582 [58].

The few relations found between seedling resistance and late heading could be due to late genotypes that progress better in rainy zones in Spain with moderate temperatures, where rust attacks are more frequent. This result agreed with the greater resistance and late heading of domesticated emmer wheat (181 days vs. 177 and 173 for rivet and durum wheat, respectively). Other authors have also detected a positive influence of days to heading on the resistance to stem rust in common wheat [59].

The underlying hypothesis for the associations between ecogeographic conditions and resistance to pathogens was that certain types of environments would favour the emergence of disease resistance within in situ populations of landraces. The associations between resistance traits and ecogeographic variables of the collection site can help to identify those environments that are likely to impose selection pressure for the emergence of resistance genes. In the present study, the analyses of 75 ecogeographical variables indicated that there are significant differences between the origin sites of the resistant and susceptible accessions of durum and rivet wheat. These differences were mainly related to thermal variables of the site for CDJ13 (isothermally), and to both thermal and hydric variables for JDJ15. For the latter isolate, resistant genotypes most likely came from zones with a uniform temperature, avoiding thermal stresses in the hottest season, and with higher rainfall from October to February. The importance of these bioclimatic differences between the origin sites of resistant and susceptible groups were also shown with the random forest classification and in adult plant in the field evaluations. Furthermore, the mean values of these ecogeographic variables in the resistant/susceptible groups were similar at both growth stages (Tables S4 and S7). These results were again consistent with the higher resistance of domesticated emmer wheat since the origin sites of this subspecies (mainly northern Spain) had the highest mean temperature uniformity, and October and November precipitation (Table S10). The east of the country (Valencia Community and Balearic Islands) included more resistant durum and rivet wheat accessions to the durum wheat isolates at seedling stage than the other zones. It is known that this Spanish region is a favourable zone for leaf rust occurrence [60]. Although this result could not be confirmed at the adult stage in the field trial, this agroecological zone is characterised by having uniform temperatures and higher October precipitation, in agreement with other studies that also found significantly higher numbers of durum wheats resistant to stem rust than expected in the Spanish eastern coast [59].

As a general rule, leaf rust uredospores needs a temperature around 20 °C and a high humidity for at least three hours (usually before dawn and resulted in dew deposition on the leaves) to start an asexual cycle [3]. Our results revealed a higher influence of temperature

uniformity than hydric variables for both durum wheat isolates, which confirmed that pathogens respond more to the diurnal temperature variation than to other ecogeographic variables [30]. Some authors have also found stronger associations between rust severity and thermal variables than with hydric variables in common wheat [61,62], whereas other studies pointed out the importance of the rainfall within the growing season [63]. In our study, the rainfall in autumn and winter (from October to February) was positively associated with resistance to JDJ15. These months correspond to the growth period from the first leaves to the end of tillering. After the dry summer period, October and November are the first months the precipitation increases (Figure S2). Winter conditions also affect the survival of the primary inoculum of leaf rust [62,64]. The humidity is also relevant at the end of winter (February), when the temperature increases (Figure S2), resulting in a higher rust severity in the origin site. Other studies in common wheat have reported that leaf rust is associated with warm, rainy growing seasons [65,66]. In our study, the variables related to temperature uniformity and maximum values were more relevant for both isolates. This could be due to the other studies referred to common wheat, grown in colder zones where low temperatures can reduce leaf rust development, whereas high temperatures can be a more relevant limiting factor in durum wheat growing zones. The lack of relation between resistance to the common wheat leaf rust isolate and the origin site of the landraces is expected as common wheat isolates are not specialized to infect durum wheat [7], and the pathogen pressure of this kind of isolates was probably low in the zone of origin for the development of an adaptive resistance.

The relations between resistance and some ecogeographic variables of the collection site at both seedling and adult stages indicated that landraces from some areas could provide genotypes more resistant to some durum wheat isolates from Spain. Several studies [67–69] have shown that genetic variation for resistance to pests and diseases can be detected in germplasm originating from locations with an environmental profile similar to the collection sites of a reference set of accessions with known resistance. In the present study, two predictive models for leaf rust resistance were elaborated for the durum wheat isolates based on the seedling test scores of the core collection and the bioclimatic variables as the predictors. The goal of the germplasm testing program is to postulate which genotypes have seedling rust resistance genes and to determine if these genotypes are resistant in the field. In durum wheat, almost all resistance genes are effective through all plant stages (seedling resistance genes), with some rare exceptions such as the landrace Gaza that carries one adult plant and one seedling resistance gene [18]. Thus, both models were used to predict the resistance reaction of adult plants since this is the one disease response relevant for breeding. Although the development and validation of the models were based in leaf rust scores recorded at two different growth stages, the results indicated that both models had a high accuracy and agreement between predictions and disease resistance at adult stage. The correct classification score with the models was higher than 89% and sensitivity values were of 80%. Other studies have shown that prediction models based on ecogeographic variables is an efficient approach for the identification of rust resistant accessions [28–31]. These studies based on adult plant scores from different environments reported sensitivity values between 65 and 74% [28,29,31] and correct classification about 77% [29,31]. The better performance of the models developed in the present study could be due to the environmental conditions of the field trial are specific of the isolate attacks (the same province for CDJ13 and the same locality for JDJ15). It has been reported that different screening conditions between the sets for develop and evaluate the model can introduce variation in the trait scores that would be interpreted as noise by the classification algorithm. [29] Thus, the environmental conditions in which the evaluation data for the development of the model are obtained may influence the predictive power of the models. In the present study, this influence could be low since the data used to build the model were obtained under controlled conditions in the greenhouse.

## 5. Conclusions

Sustained gains in plant breeding rely on the amount or variation in crop gene pool. Identification of sources of novel diversity for leaf rust resistance in germplasm is an essential pre-breeding activity to mobilizing novel alleles to elite varieties. In the present study, several resistant landraces were identified that potentially may be used in durum wheat breeding programs, especially durum wheat accessions (e.g., BGE045670 Colorado de Cabra for hypersensitive resistance, and BGE047515 Claro de Balazote for partial resistance) but also rivet wheat (BGE047509 Blanco de Vegadeo), and domesticated emmer (BGE048901 Escaña doble).

The relevant relationships between the response to leaf rust and the collection site environment of Spanish landraces, both at seedling and adult stage, detected in the present work is a very useful information to develop smaller subsets for future screening against new leaf rust races with a higher hit rate for rust resistance. Development of two predictive models for leaf rust resistance based on seedling test scores and bioclimatic variables as predictors can be a very valuable tool for an optimal exploitation of genetic resources by durum wheat breeders. Furthermore, molecular analysis of resistant accessions could identify alleles for leaf rust resistance more likely to be found at similar eco-geographic environments. These results provide an opportunity for breeding programs in Spain and elsewhere to target their crossing and selection activities so they can yield lines with effective rust resistance in the Mediterranean region.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2077-0472/11/4/277/s1>, Figure S1: Pearson correlations between disease severity in the field and the disease parameters infection type (IT) assessed for each isolate Conil Don Jaime 13 (CDJ13), Jerez Don José 15 (JDJ15) and Peralta García 14 (PG14). Coefficients  $> |0.2|$  are significant ( $p < 0.05$ , Figure S2: Mean values of monthly rainfall (prec), and mean (tmean), minimum (tmin) and maximum (tmax) temperature of the locations of the durum and rivet wheat accessions, Table S1: Agronomic variables recorded in each accession, Table S2: Ecogeographic variables considered in the characterization of the durum wheat accessions obtained from various sources compiled by CAPFITOGEN [36], Table S3: Disease parameters infection type (IT), disease severity (DS) and leaf necrosis (LN) of three leaf rust isolates inoculated on the 94 landraces of the core collection of tetraploid wheat of CRE, Table S4: Means of the ecogeographic variables (see Table S2) ordered according to their significant differences ( $p < 0.05$ ) between resistant and susceptible accessions at the seedling stage for durum and rivet wheat and the durum wheat isolates CDJ13 and JD15, Table S5: The top 30 bioclimatic variables (see Table S2) selected according to the mean decrease accuracy of the random forest approach for durum and rivet wheat within each durum wheat isolate at seedling stage, Table S6: Disease Severity evaluated in adult plant stage in field plots, Table S7: Means of the ecogeographic variables (see Table S2) ordered according to their significant differences ( $p < 0.05$ ) between resistant and susceptible accessions based on their Severity values at adult stage in field plots for durum and rivet wheat, Table S8: Disease parameters infection type (IT) and uredinium size (UrS) of the leaf rust isolate Conil Don Jaime 13 assessed in 74 susceptible accessions ( $IT \geq 7$ ) of the core collection of tetraploid wheat of CRE, Table S9: Pearson's correlation ( $p < 0.05$ ) between uredinium size and ecogeographic variables (see Table S2) in the tetraploid accessions ( $n = 40$ ) evaluated for partial disease resistance to the CDJ13 isolate at seedling stage, Table S10: Mean values and standard deviations for each subspecies of the most relevant ecogeographic variables (see Table S2) significantly different between resistant and susceptible groups.

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**Data Availability Statement:** The data presented in this study are available within the article and the supplementary material.

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