


Higher rust resistance and similar yield of oat landraces *versus* cultivars under high temperature and drought

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Accepted: 30 November 2016 / Published online: 20 December 2016
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Abstract Oat crop is not particularly well adapted to hot and dry weather, as shown by a 3-fold yield reduction in Mediterranean compared to Northern regions. As a consequence, there is a need to identify more resilient oats adapted to current Mediterranean and future climate conditions. Here, we studied the performance of oat landraces under Mediterranean conditions, including the resistance to their most devastating disease, the crown rust. One hundred forty-one genetically characterized Spanish landraces were evaluated over two crop seasons at four contrasting locations in Spain and Egypt. Genotype-environment interactions were studied using heritability-adjusted genotype plus genotype-environment biplot analyses. The impact of climate variables on agronomic traits and the adaptation of particular landraces to environmental factors were inferred from non-metric multivariate scaling and canonical correspondence analyses. Results show an average oat landrace grain yield of 1500 kg/ha, which is similar to the mean yield of commercial varieties. Nonetheless, commercial varieties had 20% higher harvest index than landraces, which is explained by the higher biomass of landraces. Moreover, oat landraces showed high levels of rust resistance with mean values of the area under the disease curve of 2.58, which is approximately 25% lower than

that of commercial varieties. Furthermore, several landraces carried a broad spectrum type of resistance which is expected to be more durable and efficient against different rust isolates.

Keywords *Avena sativa* · Breeding · Crown rust · Genotype × environment interaction · Landraces

1 Introduction

Oat (*Avena sativa* L.) is an important cereal crop cultivated for grain, feed, fodder and straw over approximately 9 million ha globally (FAO 2015). *A. sativa* is the main cultivated oat species. It includes the white oats, preferred for milling and used for human food and fodder, and red oats (formerly known as *Avena byzantina* K. Koch) preferred for hay (Stevens et al. 2004). During the last 20 years, the oat cultivated area in the Mediterranean rim has steadily increased by approximately 7500 ha per year (FAO 2015). This is in part due to the good oat adaptation to a wide range of soil types, and because on marginal soils, oats can outperform other small-grain cereals (Stevens et al. 2004). This almost equalled the oat cultivated area between Northern Europe and Mediterranean rim. However, the oat yield in Northern Europe remains much greater than that of the Mediterranean area (approximately 2.7-fold; FAO 2015). This highlights that there is still plenty of possibilities to increase oat yield by improving crop resilience to southern conditions.

Some of the reasons for the lower yield observed in Mediterranean regions might be the agro-climatic differences between Northern and Southern regions (i.e. water availability or temperature) and the limited adaptation of oat cultivars to these latter agro-climatic conditions. Indeed, the cultivars grown in the Mediterranean rim are usually spring cultivars bred in northern countries used as winter crop. Therefore,

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there is a need to study the adaptation of oat germplasm to Mediterranean agro-climatic conditions, which are characterized by mild and moderately rainy winters and warm and dry springs, and to implement specific breeding programmes based on the particular requirements of the southern areas (Sánchez-Martín et al. 2014).

One of the challenges to be tackled is the poor oat adaptation to high temperatures and drought, common to most Mediterranean growing areas. Although oats have high soil nutrient use efficiency due to their vigorous root systems that exploit the soil well, their transpiration rates and, hence, water requirements are higher than that of other small grain cereals (Ehlers 1989). Thus, oats are especially susceptible to grain abortion caused by drought and high temperatures, showed as empty white spikelets (Fig. 1). Beside these abiotic stresses, one of the main factors responsible for yield losses and yield instability is the oat susceptibility to diseases. It is thus crucial to identify sources of disease resistance (Prats et al. 2015). Resistance to crown rust is one of the most desirable traits, since this disease (Fig. 1) causes high losses in yield and grain quality (Simons 1985) especially in the Mediterranean rim where rust populations are more virulent than in the centre and north of Europe (Herrmann and Roderick 1996). The use of race-specific genes for resistance (*Pc*) has been the primary means of control. Up to date, more than 90 genes for crown rust resistance have been assigned with permanent designations (Chong et al. 2000). Unfortunately, these genes are rapidly defeated by the emergence of new pathogenic populations. The gene *Pc94* transferred from *Avena strigosa* is currently regarded as the most effective gene for resistance to *Puccinia coronata* (Chen et al. 2007). However, virulence against this gene, albeit at low frequency, has already been detected in Canada and in the Mediterranean area (Chong et al. 2011; Sánchez-Martín et al. 2012). Breeding of resistance varieties is considered one of the most effective, economical and environmentally friendly control method (Stevens et al. 2004). However, most of the modern oat cultivars currently used in the Mediterranean rim are not particularly resistant to rust, since this disease is favoured by hot and dry climate. Thus, novel and durable resistance sources are needed.

Plant landraces are domesticated, regional ecotypes or locally adapted species that has developed over time through adaptation to its natural environment. Therefore, they are adapted to local climatic conditions, cultural practices, disease and pests (Harlan 1975). During the twentieth century, landraces have often been replaced by modern cultivars contributing to the genetic stability of several crops including wheat, barley and maize (revised by Newton et al. 2010). Although these modern cultivars may be higher yielding under high input systems, landraces have considerable potential under low input systems which are usually the characteristics of the Mediterranean rim. Landraces might also be good reservoirs of favourable traits related to disease resistance, such as rust resistance, abiotic stress tolerance and, in general,

adaptation to environment. Transfer of beneficial traits from landraces to modern cultivars is relatively straightforward, since there is no barrier to crossing. Landraces are thus valuable source of new allelic diversity for breeding programmes and ideal candidates to test for oat adaptation, agronomic performance and disease resistance under variable environments.

However, selection of agro-climate adapted genotypes is not so straightforward since they are strongly affected by the genotype \times environment interaction (GEI). Given the difficulty of selection for agronomic traits, multi-environment yield trials (MEYTs) are used to identify both superior genotypes and best locations for selection. GEI attenuates the association between phenotype and genotype, reducing genetic progress in plant breeding programmes. Gaining knowledge on how GEI affects genotypes' performance may aid in selecting the best genotypes and defining their optimum environments to maximize yield. The ANOVA is an additive model that describes main effects accurately and determines whether GE interaction is a significant source of variation, but it does not provide insight into the patterns of genotypes or environments that give rise to the interaction. To solve this problem, several additional statistical analyses have been developed. The additive main effects and multiplicative interaction (AMMI) models and genotype plus genotype \times environment (GGE) biplot analyses are between the most frequently used (Gauch et al. 2008). In particular, GGE analyses have been previously proven useful to characterize disease resistance and to select breeding material for yield stability and other agronomic traits in field trials in oats (Sánchez-Martín et al. 2014) and other species (Villegas-Fernández et al. 2009; Fernández-Aparicio et al. 2012; Rubiales et al. 2012, 2014; Flores et al. 2012, 2013).

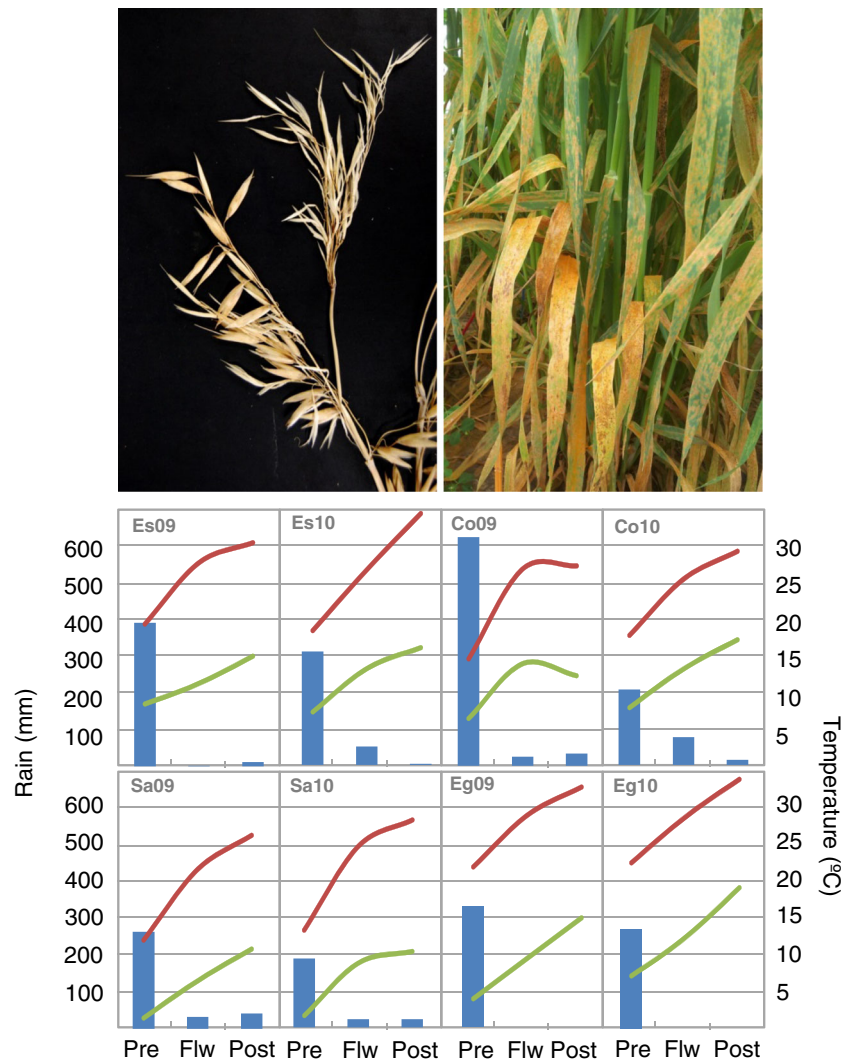
In this work through multi-year, multi-site trials involving a collection of 141 white and red oat landraces and GGE approaches, we aim to provide the theoretical basis to test oat performance in Mediterranean environments. Furthermore, we aim to determine (1) core testing sites for selection; (2) best adapted landraces to Mediterranean agro-ecological conditions, with special attention to yield stability and resistance to its most devastating disease, the crown rust; and (3) climatic variables that impact most on the different oat agronomic traits in a scenario of climate change.

2 Materials and methods

2.1 Plant material and experimental design

An oat network consisting of 141 white and red oat landraces, provided by "Centro de Recursos Fitogenéticos", INIA, Madrid, was evaluated over two crop seasons at three contrasting locations in Spain for agronomic traits and four locations for rust resistance in Spain and Egypt. Climatic data of the different environments (combination of a year and location)

Fig. 1 Upper panel shows the main constraints for the oat crop under Mediterranean environments: on the left hand side, the picture shows an oat panicle with numerous aborted spikelets due to low water availability and high temperatures; on the right hand side, the picture shows an oat plant heavily infected with the crown rust (*Puccinia coronata* f. sp. *avenae*). The lower panel shows several climatic variables that characterize the Mediterranean environments studied. Cumulative rainfall (bars) and average maximum (red line) and minimum (blue line) temperatures during pre-flowering (Pre), flowering (Flw) and post-flowering (Post) periods for the different studied Mediterranean environments: Es, Escacena; Co, Cordoba; and Sa, Salamanca in Spain; and Eg, Kaf-el-Sheik in Egypt. The number following the location indicate the year



are displayed in Fig. 1. A list of the landraces evaluated together with other relevant data from GenBank and details of the genetic relationships between these cultivars has been previously reported in Montilla-Bascón et al. (2013, 2015). Briefly, genetic studies distinguished one group of red oats and two groups of white oats. The red oat group included also four landraces described as white oats, the genotypes 139, 64, 27 and 106, because they shared a 235 pb allele exclusive to this group. The two groups of white oats differed mainly in the adaptation to altitude. Thus, one group contained landraces adapted to high altitude and their associated cooler temperatures and probably poorer soils than the other landraces adapted to low altitude.

At each location, a randomized complete block design with three replicates was used. Each replicate consisted in independent plots consisting of three 1-m-long rows bordered by the rust-susceptible oat cultivar Cory with the aim of providing the most appropriate conditions for the disease development. Within each plot, the rows were separated from each other by

30 cm at a sowing density of around 90 seeds m^{-2} . Spanish trials were performed in three contrasting locations (Escacena with 88 m altitude and light clay eutric vertisol; Cordoba with 90 m altitude and light clay calcic cambisol; and Salamanca with 829 m altitude and sandy loam or sandy-clay-loam vertic luvisol soils, respectively) during growing seasons 2009–2010 and 2010–2011. Sowings took place between October and December, according to local practices, except in Cordoba during the season 2010–2011, in which, due to intense rain levels, the sowing took place in January. Egyptian trials were carried out in a single location, Kafr El-Sheikh with 8 m altitude, over the growing seasons 2007–2008 and 2008–2009 on a loamy calcareous fluvisol according to FAO (2011). No irrigation was performed in Spanish trials, but Egyptian plots were level basin flood irrigated according to local practice. This was done at sowing and then on the 1st of February and 1st of March by the application of 800 $m^3 ha^{-1}$ each time. No artificial inoculation was performed at any location, crown rust infection occurring naturally. Hand weeding was carried

out when required, and no herbicides or fertilizers were applied. Trials were hand harvested.

2.2 Seed yield, biomass, earliness and disease assessments

At maturity stage, total aboveground dry matter was determined following field-drying of the plant material for at least 1 week. All grain was oven-dried at 70 °C. Yields are presented on an oven-dry basis of seeds weighted (kg/ha). Biomass data are based on the aboveground plant weight (kg/ha), and harvest index (HI, %) was calculated as the ratio between grain weight to total dry matter. Earliness was estimated as growing degree days (GDDs). The baseline temperature of 5 °C was used to calculate GDDs. The GDD for each day was calculated as average of maximum and minimum daily temperatures in degree Celsius minus 5 °C. When disease symptoms were observed, disease severity was assessed as a visual estimation of the percentage of whole plant tissue covered by crown rust pustules. Observations were made weekly from disease onset to the end of the disease cycle. This allowed calculation of the area under the disease progress curve for rust disease (rAUDPC) according to Wilcoxson et al. (1975).

2.3 Statistical analysis

A combined ANOVA for randomized complete-block designs was carried out using SAS® 9.3 (SAS Institute Inc.). Prior to each ANOVA, tests for normality and equality of variance were conducted for each dependent variable. For each location-year, the genotypic variance (σ^2_g) and error variance (σ^2_e) were estimated using Proc Varcomp of SAS® 9.3 (SAS Institute Inc.). Arcsine transformations of data not conforming conditions of normality and homogeneity (i.e. HI data) were performed to conform to the ANOVA assumptions. *F* ratios, used to test effects for randomized complete block experiments combining location-year environments, were determined according to McIntosh (1983). Both genotypes and environments were treated as random effects. Pearson correlations were calculated to detect statistical correlations between trait measurements.

We here used the HA-GGE biplot (Yan and Holland 2010; Sánchez-Martín et al. 2014) since it takes into consideration any heterogeneity among environments by giving weights to the test environments proportional to their root square heritability. Therefore, it is most appropriate for visual evaluation of the test environments and genotypes. Analyses were made with the SAS® 9.3 (SAS Institute Inc.) programme developed by Burgueño et al. (2003) to graph GGE biplots. The target environment axis (TEA) is represented by a straight line drawn through the biplot origin and the *average environment*, which was defined by the mean ordinates of all environments in the biplot. Genotypes located on the polygon vertices reveal

the best or the poorest for a particular environment. Data derived from biplots were tested statistically by non-parametric bootstrapping to construct 95% confidence intervals on the basis of empirical distributions of estimated parameters. Because standard variance deviation needs to be estimated on a balanced data set, we randomized (with replacement) only either columns or rows (but not both), keeping the other fixed (Yang et al. 2009). This resampling process was repeated 1000 times to provide accurate estimates of confidence intervals.

To evaluate the influence of environmental factors on the agronomic traits, 10 climate variables (maximum temperature, minimum temperature and rain during pre-flowering, flowering and post-flowering period and photoperiod length during flowering time) obtained from the AEMET database for each location were subjected to non-metric multi-dimensional scaling ordination (NMDS; Anderson 2001). This ordination technique is well suited to handle non-normal and non-continuous data (McCune and Grace 2002) and allowed us to reduce the climate variable matrix prior to model each agronomic trait. Canonical correspondence analysis (CCA) was performed to determine the relative impact of the selected climatic variables on agronomic traits and genotype performances. Analyses were made by PAST software (Hammer et al. 2001).

3 Results and discussion

3.1 Global means for environment and overall influence of climatic variables on agronomic traits

Differences were observed for each agronomic trait among environments. Both yield and biomass showed an important variation among the different environments with variation coefficients of 58.4 and 39.1, respectively. A similar study was performed on cultivars in same environments (Sánchez-Martín et al. 2014) showed variation coefficients of around 15%. This highlights the high variability of landraces, which could be expected, since as stated in their definition, “they are often highly variable in appearance” (Harlan 1975). Interestingly, the overall yield of the oat collection across the different environments was similar to the yield of the five most cultivated varieties in Spain currently (i.e. Aintree, Caleche, Chapline, Cory, Fringante and Orblanche) (Sánchez-Martín et al. 2014). However, varieties had a 20% higher HI than landraces due to the higher biomass of landraces. This may reflect the effort that plant breeding programmes have paid over the last years to improve HI of modern varieties (Gepts 2004). Biomass, HI and, particularly, yield were positively influenced positively by high rain levels during post-flowering period and negatively by high maximum temperatures, since their vectors followed opposite directions, in

particular those of temperature with those of rain during post-flowering period (Fig. 3, up-left panel). Yield was the trait most influenced by water availability and maximum temperatures during the post-flowering period with vectors almost in 180°. Indeed, the highest yield was observed in the location of Salamanca, which was characterized by the lowest maximum and minimum temperature over the season, relatively low levels of rain during pre-flowering and higher rain levels during post-flowering period (Fig. 1). Although all tested locations are within the Mediterranean agro-climatic zone (Iglesias et al. 2012), Salamanca is located in Mediterranean north region, whereas Cordoba and Escacena are located in the Mediterranean south region (Iglesias et al. 2012). In the latest, high temperatures coupled with low water availability at flowering and grain filling (Fig. 1) could have influenced the lower agronomic performance observed, increasing the number of aborted spikelets and, hence, reducing yield. The lowest yield was observed in Co10, while in the other environment of this location, Co09, landraces showed both high yield and biomass. This suggests that the poor yield observed in Co10 could be due at least in part to the harsh climatic conditions of the autumn 2010 at Cordoba, in which very intense rains distributed in several weeks compelled to delay the sowing date up to end January, reducing the GDD to the lowest value of this location (626.3). Indeed it was observed a positive statistical correlation between yield, biomass and HI. The highest significant correlation was found between yield and biomass ($r = 0.7$, $P < 0.001$). A significant correlation was also found between flowering and yield ($r = 0.4$, $P < 0.001$) and flowering and biomass ($r = 0.55$, $P < 0.001$).

Data showed that although flowering (GDD) was influenced by genetic factors, here, it was more strongly affected by environmental parameters. The variation coefficient of GDD was 10.8, and the sum of squares corresponding to

“environment” factor was almost 90% of that of the model (data not shown), indicating higher variability between environments than within them. For instance, flowering was highly influenced by high temperatures (both maximum and minimum) and photoperiod with inverse trends (Fig. 3 up-left panel). The data indicated that once plants had accumulated the minimum necessary GDD, the decisive factor for flowering was photoperiod. Thus, plants started flowering when daytime reached 13 h 35' in Cordoba, even when sowing had been delayed by almost 2 months in Co10. Oat plants started flowering with 13 h 52' daytime in Escacena that had less daylight period than Cordoba for a same date, allowing plants to accumulate higher GDD. In Salamanca, which presented the lowest temperatures, plants started flowering when daytime reached 14 h 35' (Sa09) and 14 h 39' (Sa10), being this location the one in which plants accumulated the lowest GDD. The high influence of photoperiod observed here is in agreement with reports in other species demonstrating that after vernalization requirements, photoperiod and seasonal changes in photoperiod are the most important environmental cues impacting on the timing of flowering during the course of a year (Jackson 2009). Our data shows that despite the moderately mild winter, all landraces fulfilled their vernalization requirements. This, together with the significant positive correlation between GDD and yield/biomass, suggests that relatively earlier sowings in autumn allow longer periods to flower and generally favour higher biomass and yield. Taking these data into account, it is not surprising that the landraces GDD was similar to that of the most cultivated varieties, with overall means of 1005.1 and 1018.4, respectively, for landraces and varieties (Sánchez-Martín et al. 2014).

In relation with yield and biomass parameters, we also assessed the harvest index (HI) which also showed a high variation among environments (Table 1). The harvest index

Table 1 Global means and GE interaction for yield (kg/ha), biomass (kg/ha), H Index, flowering (growing degree days) and rust severity (rAUDPC) for each environment for the multi-environment study

Environment	Yield	Biomass	H index	Flowering	rAUDPC
Global means					
Co09	1197.7	6161.8	0.160	1035.7	1.3
Co10	333.0	2089.2	0.128	626.3	3.1
Es09	526.2	4411.6	0.099	1408.1	5.6
Es10	648.5	5374.6	0.098	1202.9	2.2
Sa09	1987.3	5162.1	0.270	791.1	0.5
Sa10	1717.3	9743.5	0.139	966.7	2.8
Eg07	ND	ND	ND	ND	5.9
Eg08	ND	ND	ND	ND	5.8
G × E interactions					
% Explained variation E	52.72	59.4	59.13	86.50	31.55
% Explained variation G	13.11	8.10	16.94	4.55	27.70
% of PC1 + +PC2	44 + 22	35 + 25	44 + 17	50 + 17	58 + 18
G + GE/(E + G + GE)	0.46	0.38	0.41	0.13	0.66

Es Escacena, Co Córdoba, Sa Salamanca, Eg Kafr El-Sheik

is the ratio between grain yield and total biomass and reflects partitioning of photosynthate between grain and harvestable product (Hay 1995). Hence, it is an important parameter for crop production, and its improvement is one of the greatest achievements that differentiate commercial varieties from their wild ancestors (Gepts 2004). High HI values mean that the allocation of carbon is directed to grain instead of biomass production and can be considered as a good trait in breeding high-grain yielding cultivars. Data showed a strong correlation between grain yield and HI ($r = 0.7$, $P < 0.001$), whereas a significant but very small correlation was observed between HI and biomass ($r = 0.09$, $P < 0.001$).

Rust incidence was among the most variable parameters with a variation coefficient of 52.9. The variability was observed both between locations and between the different environments of a location. It was closely associated with temperature and rain vectors, in particular during post-flowering period (Fig. 3, up-left panel). This explains the high incidence of rust in Mediterranean rim that generally has high temperatures and moderate rain levels during the pre-anthesis period, which are the best conditions for rust development (Fig. 1). The highest rAUDPC was observed in both environments of Escacena and Egypt that follow that premises. As expected, rust severity was negatively correlated with yield ($r^2 = -0.3$; $P < 0.001$). This highlights the importance of improving oats for rust resistance in the Mediterranean rim. Indeed, rust populations have been reported to be more virulent within the Mediterranean area compared with northern regions, which is also associated with the favourable climatic conditions for their development and spread (Herrmann and Roderick 1996). Rust resistance improvement is not fulfilled for most currently used varieties that have been bred in northern regions in which rust resistance may not be a priority. Accordingly, the landrace collection has a mean rAUDPC 24.5% lower than most of the varieties currently cultivated in Spain (Sánchez-Martín et al. 2014). This reflects a higher rust resistance of the landraces and highlights the potential of the collection for future oat breeding for rust resistance, which is crucial for a sustainable oat cropping system in the Mediterranean.

3.2 Variances analyses

Significant differences ($P < 0.0001$) for genotype (G), environment (E) and genotype by environment (GE) interaction were detected for all agronomic traits. For yield-related traits, such as grain yield, biomass and HI, E accounted for nearly 60% of the explained variations, leaving only a small weight of ca. 15% for G alone (Table 1). This highlights the importance of selecting elite genotypes in multi-site, multi-year trials. In a previous study, the weight of G of currently used elite varieties in respect to the explained variation was much higher, increasing up to near 30% (Sánchez-Martín et al. 2014). This highlights the breeding efforts made in these

varieties for high yielding traits. Flowering showed the highest weight of the E, accounting for approx. 95% of the explained variation. By contrast, rust severity showed the highest weight of G, indicating large genetic variability for this trait in the landrace collection and a weaker interaction of the resistance response within the environments tested compared with other traits.

Variance analyses provide a general overview of variation and detect potential GE interactions. However, it is not useful to generate information about trends that may arise by interactions or to create a viable method to select stable genotypes. Then, we used GGE analysis combining ANOVA and principal component (PC) approaches to graphically display G and GE interactions and to identify candidate genotypes with desirable and consistent performance across years and locations for each trait. Several recent reviews have compared and contrasted two of the most used analyses to study GE interactions, AMMI and GGE, with respect to their suitability for GE analysis (i.e. Yan and Tinker 2006; Yan et al. 2007; Gauch et al. 2008). These highlight the suitability of both methods as long as they justify model diagnosis and use confidence regions to make critical decisions for genotype selection based on statistical tests (Yang et al. 1996).

Here, we used HA-GGE biplots to infer both the utility of the environments in terms of selection response and the behaviour of the cultivars in these different environments (Yan and Holland 2010). In relation to model diagnosis, we used a rank-two approximation. This means that biplots were constructed using the scores derived from the first two PCs to approximate the information content of the two-way GE table. To do this, it is important for these first two PCs to capture a high percentage of the total variability otherwise the patterns identified may be inaccurate or unreliable. According to Yang et al. (1996), the first two PCs should account for approximately 60% of the (G + GE) variability. Our data show that the first two principal components explained between 60 and 82% of total G + GE interaction for all traits (Table 1). In addition, the combined (G + GE) effect should account for >10% of the (E + G + GE) variability, which was observed for all traits. In relation with the other points to be taken into account, we carried out bootstrapping of the data according to Yang et al. (1996) and constructed 95% confidence intervals to support the information derived from the biplots generated as stated in materials and methods.

3.3 Test of environmental evaluation

Identification of suitable locations for testing segregating populations and collections within breeding programmes is one of the key factors for breeding success. Main characteristics of suitable locations are the capability to discriminate among genotypes and the representativeness of the average environment to the targeted area to ensure that the selected genotypes

have the desired adaptation and repeatability necessary to ascertain their good performance in the coming years. According to Yan and Holland (2010) in the HA-GGE biplots, the vector length of an environment is indicative of its discrimination power; the cosine of the angle between an environment and a TEA_a (average environment) or between two environments is an indicative of the representativeness and repeatability, respectively, while the vector projection onto the TEA_a is an overall measure of the usefulness of an environment.

Focusing on grain yield (Fig. 3), Cordoba together with Escacena, characterized by relatively high maximum and minimum temperatures over the season and scarce water availability during grain filling, showed a high representativeness of the average environment and were the most useful locations for selecting superior yielding genotypes (as indicated by their longest projections over the TEA_a). Among these, Cordoba showed higher repeatability than Escacena according to the small angle between years (lower than 30°), and therefore, it could be considered ideal for selecting superior genotypes. The two environments corresponding to Salamanca location, Sa09 and Sa10, showed high repeatability indicated by the acute angle between both and high representativeness of the average environment. However, their small projections on the TEA_a suggested that this location would not be useful to discriminate among genotypes. From the climate point of view, the mild temperature and higher water availability during grain filling in this location favoured the highest yield and lowest variation within the landrace collection, which make the selection of outstanding genotypes more difficult.

Biomass production, HI and flowering (GDD) (Fig. 3) followed a trend similar to that of yield, supporting the good correlation observed between yield, biomass and HI. All environments indicated a good representativeness of the average environment, although Cordoba and Escacena showed the highest representativeness of the average Mediterranean environment, indicating their suitability for selection for these traits. Interestingly, after bootstrap analysis of flowering data, the 95% confidence interval graph showed the smallest intervals, probably reflecting the small variation observed for this trait (data not shown).

Regarding rust evaluation, the environment Sa09, characterized by the lowest minimum and maximum temperatures along the whole season, showed almost no rust infection with most genotypes showing zero AUDPC values and an AUDPC mean for this environment of 0.5. Consequently, this environment was removed for further statistical analysis. The preliminary environment-specific HA-GGE biplot for rust (rAUDPC) identified two potential mega-environments (MEs) regarding Spanish and Egyptian locations (Fig. 4), defined by Yan and Tinker (2006) as meaningful subset of similar environments. Although the number of locations in the present study was relatively low for ME definition, the results

confirmed those previously obtained on elite oat varieties for which 12 different Mediterranean environments were assessed including the Spanish and Egyptian locations (Sánchez-Martín et al. 2014). Thus, the two MEs were taken into consideration. In the present study, although all environments were positively correlated (acute angles), a clear difference was observed between Egyptian and Spanish environments (Fig. 4). Furthermore, their vectors cut the polygon at different sides. Egyptian and Spanish environments were also clearly distinguishable from the bootstrap confidence intervals estimated for the two first PC's environment scores since Egyptian location had positive PC2 values while they were negative for Spanish environments (data not shown). Thus, for further analyses, independent biplots were constructed for Spanish and Egyptian MEs, the former characterized by higher altitude, lower maximum and minimum temperatures and higher rain level.

In the Egyptian ME (ME1), analysis of the biplot showed that both environments had similar representativeness of the average environment and genotype discrimination power (Fig. 4). Regarding the Spanish ME (ME2), all locations showed good representativeness of the average environment although Salamanca with climatic conditions less favourable to rust development showed the lowest discrimination power. For rust resistance selection, Escacena, with the highest maximum temperatures over the season and moderate rain level, had the highest discrimination power showing for its two environments, Es09 and Es10, the longest projections.

3.4 Genotype evaluation

The best characteristics of ideal genotypes should be high performance and stability. These characteristics may be inferred from the biplots since projection of a cultivar over the average environment axe indicates its mean performance across all environments, and its projection over the TEA_o indicates its stability (Yan 1999). When the different environments fall in different sectors, the vertex cultivars indicate the best cultivar for each specific environment (Yan et al. 2007).

Genetic diversity of this landrace collection has recently been determined (Montilla-Bascón et al. 2013), indicating three clusters: (1) the red oats, (2) the white oats adapted to low altitude and (3) the white oats adapted to high altitude. These different clusters have been represented in the biplots in red, blue and violet, respectively, in order to extract more easily any information relating to any of the traits analysed with respect to the genetic of the collection. Vertex genotypes are represented in bold for all biplots.

3.4.1 Grain yield

Yield biplot (Fig. 3) highlighted landraces 133, 122, and 79 as highest yielding for the average Mediterranean environment.

No significant differences were detected between them according to the bootstrap analysis but they significantly differed from the other landraces. In addition, these genotypes were also among the most stable landraces across all the environments with small angles with the average environment (TEA_a). Indeed, these three landraces were among the 10% of the highest yielding genotypes in most environments according to the raw means, genotype 122 reaching the highest yield with means of 3819 and 1898 kg/ha in Sa09 and Co09, respectively. Interestingly, these three landraces showed also the highest projection on the Co09 axis characterized by the lowest rainfall conditions during grain filling, suggesting good adaptation to drought during this crucial period. This is in agreement with the ordination analysis (Fig. 3) in which this high yielding landraces (highlighted in yellow) had small (or even negative) projections on the rain post-flowering vector but high projections on temperature vectors indicating a better adaptation to this climatic conditions than the other landraces. By contrast, several landraces, such as 81, 83, 107, 66 or 62 that did not differ significantly between them according to bootstrap analysis, yielded poorly at all environments. These landraces belonged to the cluster of white oats adapted to low altitude. However, other landraces belonging to this cluster, such as 115, 87 or 20, were relatively high yielding, and the different oat clusters are homogeneously distributed in the biplot. Thus, we cannot establish any correlation between the particular genetic grouping and yield. Interestingly, these landraces (highlighted in grey in Fig. 2) were opposite in the ordination diagram to those highlighted as high yielding (highlighted in yellow). This suggests that the lowest yielding landraces were those worse adapted to high temperatures and with highest water requirements during post-flowering period (Fig. 2). Landrace 10 was particularly interesting since it showed the highest positive projections onto the maximum temperature post-flowering vector and, subsequently, the highest negative projections onto the rain post-flowering vector according to the CCA (Fig. 2). This landrace showed a good agronomic performance with positive projections on the average environments in the yield and HI biplots (Fig. 3), highlighting its resilience under extreme Mediterranean environmental factors.

3.4.2 Plant biomass

Oat straw is a valuable feed resource, more palatable to stock and more nutritious than wheat or barley straw. However, few breeding programmes are engaged in fodder oats. The common situation is thus to use cultivars bred for grain, for both grain and forage (Kirilov 2004). The HA-GGE biplot for biomass (Fig. 3) highlighted landraces 20 and 133 as the highest biomass producing landraces in the average environment. These landraces were significantly different to the remaining landraces according to the bootstrap analysis. These two

landraces were among the top 10% regarding biomass production in most environments with the highest raw means of 7450 and 7200 kg ha⁻¹, respectively. Interestingly, other landraces highlighted in the biomass biplot as good performing, such as 122, and 79, were also highlighted in the yield biplot supporting the good correlation observed between these two traits. Interestingly, except landraces 133, 79 and 134, the remaining red oats clustered on the left of the TEA_o axis, indicating the relatively poor performance of this genetic cluster for this trait. Data from ordination diagrams showed however an even distribution of the different genetic clusters in relation to the climatic variables suggesting that the lower biomass observed for red oats came from genetic factors rather than from their adaptation to specific environmental characteristic. Red oats spread in regions with frequent temperature extremes, such as the Mediterranean, southern states of USA, Africa, South America and Australia. According to our data, the poorest biomass production of red oats compared to that of white oats together with their relatively homogeneous yield distribution around the centre of the TEA_a suggests that red oats optimized the grain yield/biomass ratio during their adaptation to low water availability and high temperature conditions. Indeed, red oat HI mainly falls in the positive axis of TEA_a (Fig. 3). Interestingly, landraces with higher biomass means, showed long projections on the rain and maximum temperature pre-flowering and minimum temperature post-flowering vectors. By contrast, landraces 43, 60, 123 and 128 that had the lowest biomass means showed small or even negative projections onto these vectors (Fig. 2), highlighting the impact of these climatic variables on biomass production.

3.4.3 Harvest index

According to the HA-GGE biplot for HI (Fig. 2), landraces 93 and 123, which were statistically similar according to the bootstrap analysis, and also 21, 7 and 124 were the landraces with the highest HI with long projections over TEA_a . Among them, landrace 93 showed the highest stability (smaller angle with TEA_a). On the other hand, the white oats, 62, 81 and 83 and also 63, 70 and 107 showed the lowest HI. These landraces were also among those with lowest yield, supporting the correlation observed between HI and yield. Indeed, the landraces with highest yield had also high HI, all of them clustering on the right side of the TEA_o (Fig. 3). These low HI landraces were tightly grouped with very long projections onto the rain post-flowering vector in the ordination diagrams (highlighted in grey in Fig. 3). Thus, as for yield, these landraces relayed heavily on this environmental factor for their HI performance. Since rain during grain filling is not a characteristic of Mediterranean environments, they achieved a poor HI in this environment. By contrast, landraces 93 and 123 showed high projections onto the temperature vector and negative projections onto the rain post-flowering vector indicating

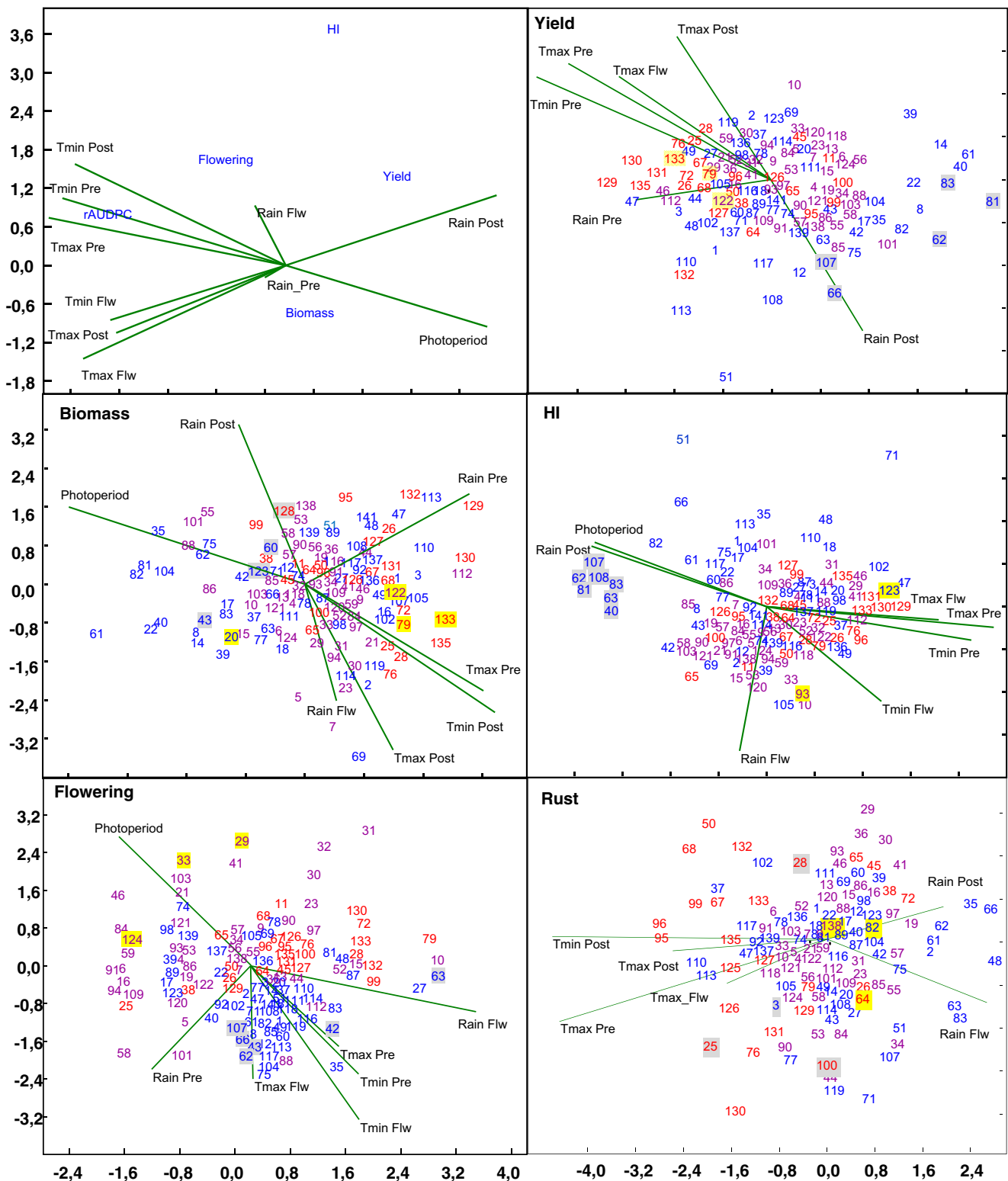


Fig. 2 Canonical correspondence analysis of 141 oat landraces and the influence of environmental factors in several agronomic traits. *Tmax*, maximum temperature; *Tmin*, minimum temperature; *Pre*, pre-flowering period; *Flw*, flowering period; *Post*, post-flowering period. Colours of the landraces indicate the genetic group with red

corresponding to red oats, blue to white oat adapted to low altitude and violet to white oat adapted to high altitude (according to Sánchez-Martín et al. 2014). Landraces highlighted in yellow and grey corresponded to landraces with higher and lower means on the average environment, respectively, according to biplot analysis

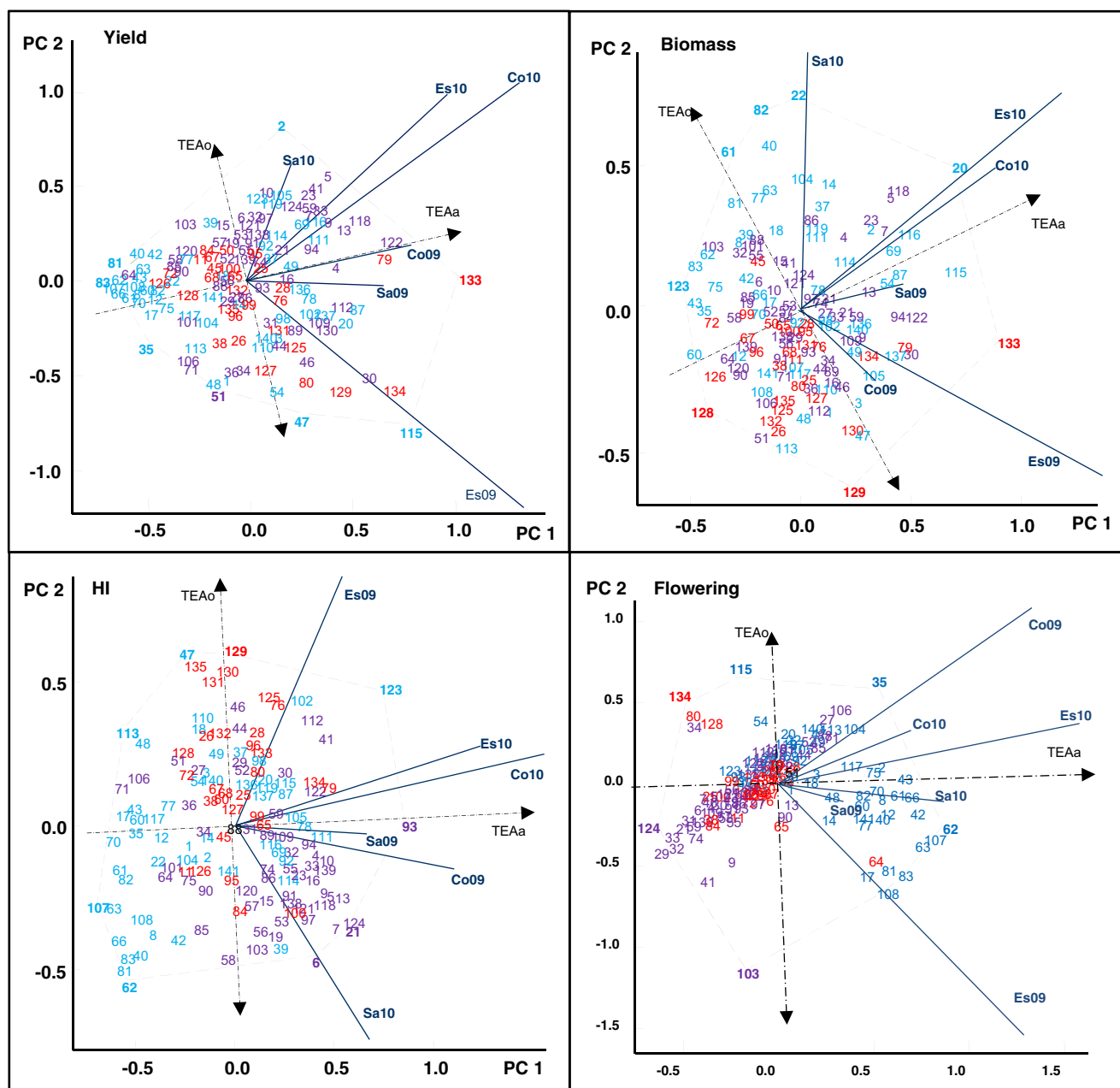


Fig. 3 HA-GGE biplot based on the grain yield (kg/ha), biomass (kg/ha), H Index (HI) and flowering (growing degree days) of 141 oat landraces grown at 6 location-year environments, from 2009 (09) to 2010 (10). *Es* Escacena, *Co* Cordoba, *Sa* Salamanca. Colours of the landraces indicate

the genetic group with *red* corresponding to red oats, *blue* to white oat adapted to low altitude and *violet* to white oat adapted to high altitude (according to Sánchez-Martín et al. 2014)

a better adaptation to the typical Mediterranean environments and, hence, higher HI.

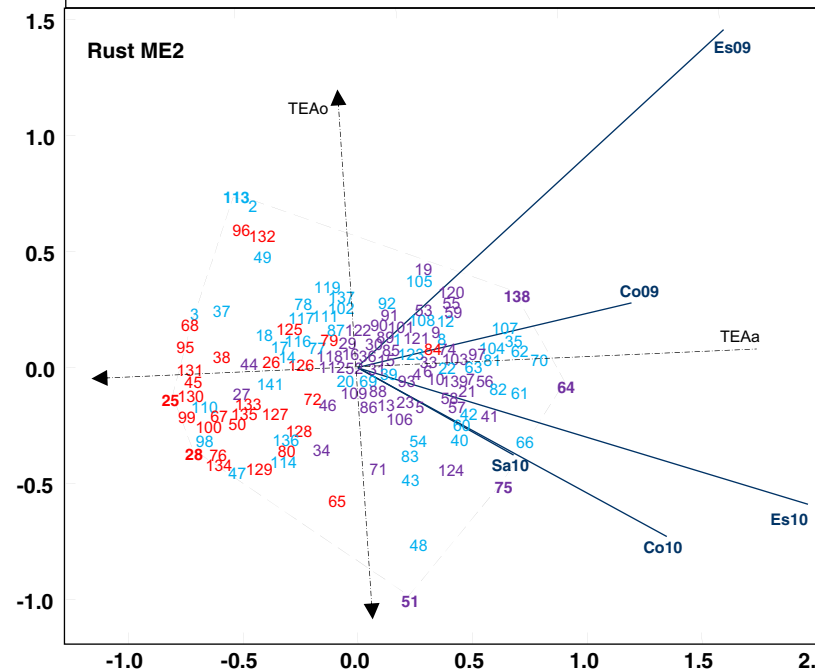
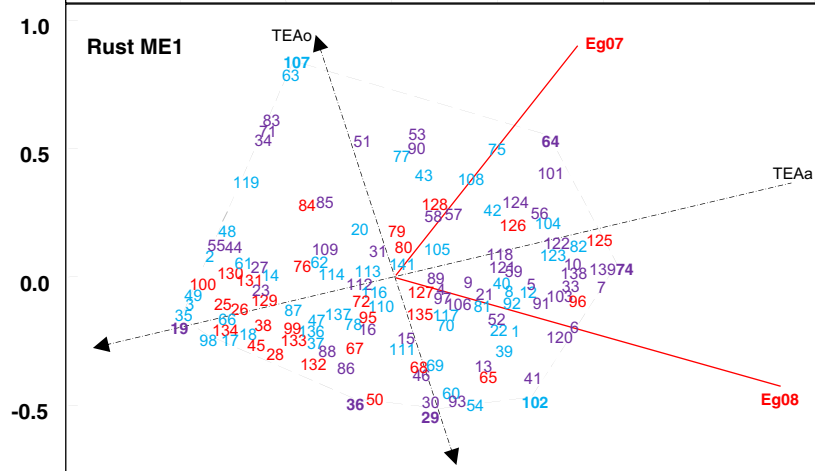
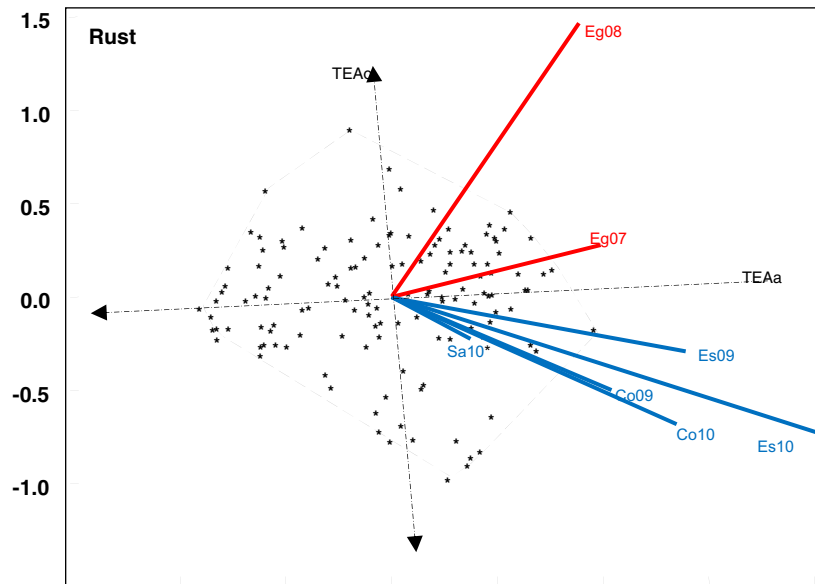
3.4.4 Days to flowering

According to the flowering biplot (Fig. 4), the earliest flowering landraces were 134, 34,128 and 80. Interestingly, we observed that most red oats clustered on the left of the TEA_o indicating early flowering in this group, with less accumulated GDD. This fact, together with their lower biomass,

supports the significant correlation found between these two traits and suggests that under Mediterranean conditions, earlier sowings which allows longer flowering period may favour

Fig. 4 HA-GGE biplots based on rust area under the disease progression curve (AUDPC) of 141 oat landraces grown at 6 location-year environments, from 2009 to 2010, including all data (*Rust*), data corresponding to mega-environment 1 including Egypt locations (*Rust ME1*) and data corresponding to mega-environment 2 including Spanish locations (*Rust ME2*). *Es*, Escacena; *Co*, Cordoba; and *Sa*, Salamanca in Spain; and *Eg*, Kafi-el-Sheik in Egypt

PC 2



higher biomass production. On the other hand, landraces such as 62 and 107, highlighted by the biplot as late flowering (according to bootstrap analysis), were also among those with lowest grain yield. The explanation might be that in the tested environments characterized for high temperatures and low rain levels, particularly during the grain filling period, a shorter cycle may constitute a way to escape for drought allowing a better performance. Figure 3 shows that most of the white oats adapted to low altitudes accumulated the highest GDD to flower. This genetic group clustered also quite tightly in Fig. 2, with positive projections onto temperature and rain vectors, opposite to photoperiod vector. This indicates that flowering of this genetic group mainly depends on temperatures, whereas flowering of the high altitude-adapted group is more dependent on photoperiod (Fig. 2). Thus, flowering of high altitude-adapted plants that usually grow under lower temperatures and accumulate lower GDD before flowering might have adapted to mainly depend on seasonal changes of photoperiod.

3.4.5 Rust infection

Since HA-biplots for rust severity indicated two MEs, genotype behaviour was analysed separately for Egyptian (ME1) and Spanish (ME2) MEs (Yan et al. 2007). The different genetic groups were evenly distributed in the biplot corresponding to ME1. However, for ME2, there was a clear cluster of red oats on the left of the TEA₀ axis, indicating that this group could be a good source of rust resistance in this ME, characterized by overall lower maximum temperatures and higher rain levels. Indeed, these two climatic variables, maximum temperature and rain, were the most important factors influencing rust severity with almost completely opposite effects (Fig. 2). By contrast, white oats adapted to high altitude clustered mainly on the right of the TEA₀ axis, indicating their poor adaptation for rust resistance. The lack of clustering in ME1 could be due to a lower pathogen pressure in this ME which might suggest the existence of different isolates in the two MEs.

Interestingly, some of the landraces, such as 61, 66 or 35, followed a different trend regarding rust resistance in the two MEs. These landraces showed high level of resistance in the Egyptian ME but low levels in the Spanish ME with mean rAUDPC values of 1.23 and 7.62, respectively, for landrace 66, for instance. Differences in the behaviour of these landraces in the two MEs point to the existence of race-specific resistance responses in them and to the presence of different rust isolates in the two ME. To trigger race-specific hypersensitive resistance, the recognition between a resistance (*R*) gene in the plant and the corresponding avirulent (*avr*) gene in the pathogen is needed. This recognition could be occurring between these plants and the Egypt ME isolate but not between the landraces and the Spanish ME isolate, triggering in the latter a virulent response. Supporting this, the rust isolate from

Córdoba overcame the resistance gene *Pc94* that was not defeated previously and was shown to be highly virulent on a set of oat differential lines (Sánchez-Martín et al. 2012). To date, most of the approximately 90 previously characterized rust resistance genes have been defeated by the evolving pathogen. This highlights the need to identify more durable sources of resistance to this devastating pathogen.

In this sense, some landraces showed stable rust resistance in the two ME. For example, landraces 3, 25, 28, 98, 100 or 134 showed low rAUDPC values in both MEs, with mean values of 1.02 and 1.05 for Spanish and Egyptian MEs, respectively. These similar trends suggest that these landraces might carry a horizontal type of resistance, such as that engaged during pre-penetration and penetration events, conferring a stable resistance over the environments studied. This type of resistance is often based on multiple and quantitative genes, and therefore, it is more difficult to be overcome by new races of pathogens compared to other resistance mechanisms based on single or qualitative genes, such as those promoting cell death (Niks and Rubiales 2002). The identification of these resistant landraces with expected durable resistance against different rust isolates and environmental conditions is of high relevance since it will allow the improvement of the oat crop in Mediterranean environments against one of its most important constraint.

4 Conclusions

Landraces have considerable potential under low input systems that are the usual conditions of oat cultivation in the Mediterranean rim. They are also important sources for adaptive traits such as resistance to biotic and abiotic stresses. However, their use must be supported by sound assessment of their adaptation through multi-site evaluation of important agronomic traits, including the resistance to important diseases across several cropping seasons. Our study based on the HA-GGE biplot method showed the behaviour of particular genetic oat groups to multi-environment trials within the Mediterranean rim and allowed the discrimination of the best and worst landraces for the different evaluated traits. These analyses were complemented with ordination analyses allowing the definition of the particular environmental factors that most influenced each agronomic trait and landrace performance. Furthermore, we identified novel and broad spectrum sources of resistance to crown rust, one of the most important constraints of oat crop in the Mediterranean rim. As far as we know, there is no previous assessment of such a large collection of genetically characterized oats for Mediterranean environments, one of the areas of higher increase in this crop in recent years. The knowledge of the productive and disease resistance features of the landraces will help to improve the

oat crop for adaptation to current and future Mediterranean growing conditions.

Acknowledgements We thank the technical staff at all sites for efficient management of the trials. This work was supported by the Spanish Ministry of Economy and Competitiveness [AGL2013-48687AGR] and regional government through the AGR-253 group, as well as the European Regional and Social Development Funds and Egyptian Academy of Scientific Research and Technology—ASRT through AECID program. JSM is holder of an FPU fellowship from the Spanish Ministry of Science and Innovation. NR is holder of a Ramón y Cajal Post-doctoral fellowship from the Spanish Ministry of Economy and Competitiveness (MINECO).

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