



Adaptation of oat (*Avena sativa*) cultivars to autumn sowings in Mediterranean environments



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ABSTRACT

Oat (*Avena sativa* L.) is a cereal widely grown as a spring crop throughout the temperate zones, being particularly adapted to areas with cool and wet summers such as Northwest Europe and Canada. There is scope for further oat expansion in Mediterranean Basin where the crop will face hot and dry weather. In this work, we assessed adaptation of 32 modern oat cultivars from different origin and usage to autumn sowings under Mediterranean agroecological conditions. Experiments were carried out over four crop seasons at 6 contrasting locations along Mediterranean Basin, including Spain, Tunisia, Egypt and Palestinian Territories. ANOVA analysis revealed genotype × environment interactions. For test environment and genotype evaluation heritability-adjusted genotype plus genotype × environment (HA-GGE), biplot analysis was performed. Biplot analysis differentiate two mega-environments one comprising the locations of Egypt and Palestinian Territories and another including Spain and Tunisia. Pearson's correlation and HA-biplots confirmed overall a positive correlation between yield and HI, and a negative correlation between yield and rust and flowering date. For other traits, relations among the traits differed depending on the ME evaluated. The study allowed determining within each ME the best discriminative location, representative of the target environment and repeatable across year and the genotypes with superior and stable characteristic for breeding of adapted oats.

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1. Introduction

Oat (*Avena sativa* L.) is an important multi-purpose cereal crop cultivated for grain, feed, fodder and straw over more than 9 million hectares globally (FAO, 2011). Oats are mostly grown in cool moist climates and they can be sensitive to hot, dry weather between head emergence and maturity. For these reasons, world oat production is generally concentrated between latitudes 35 and 65° N. Traditionally oats have been cultivated in cropping areas not appropriate for wheat, barley or maize and the cultivated area maintained stable over the years. Due to its good adaptation to a wide range of soil types and because on marginal soils oats can perform better than other small-grain cereals, there is an increasing interest to expand oat cultivation to southern countries and even to subtropical areas (Buerstmayr et al., 2007; Løes et al., 2007; Ren et al., 2007; Stevens et al., 2004; Forsberg and Reeves, 1995; Hoffmann, 1995).

In fact, autumn sown oat is increasing in Australia (Armstrong et al., 2004), south of Japan (Katsura, 2004), south China (Wan, 2004) and temperate areas of South America (Federezzi and Mundstock, 2004). These environments are characterized by mild and moderately rainy winters and warm and dry springs being winter sowing of spring crops a common practice. Thus, both grain and forage oats may be well suited to Mediterranean climates and farming systems.

Increase of *A. sativa* cultivation in these rainfed Mediterranean environments will predictably encounter water limitations as well as disease incidence such as the crown rust (*Puccinia coronata* f.sp. *avenae*). Crown rust causes high losses in yield and grain quality worldwide (Simons, 1985) but particularly in the Mediterranean Basin where rust populations are more virulent than in the centre and north of Europe (Herrmann and Roderick, 1996). Crown rust can be controlled with fungicides but this is relatively expensive and harmful due to its negative effects on human health and environment. Consequently, host resistance is being explored as the most effective, economical and environmentally friendly control method (Stevens et al., 2004). However, resistance obtained is often overcome by emerging pathogenic races. This is mainly due to the

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inappropriate use of resistance sources, of monogenic nature. Thus, it is necessary to identify novel sources of resistance that show durable resistance over time and in different environments.

Water limitation is also a feature of the Mediterranean environments. Thus, adaptation to Mediterranean conditions implies the development of drought tolerance, particularly in rainfed crops in marginal areas such as oat. The selection of adequate drought tolerant genotypes is strongly affected by the genotype \times environment interaction (GEI) (Ceccarelli, 1996). Given the difficulty of selecting drought tolerant genotypes, multi-environment yield trials (MEYTs) are used in the final selection cycles to identify superior genotypes for use in plant breeding programmes. GEI attenuates the association between phenotype and genotype, reducing genetic progress in plant breeding programmes so the knowledge of how GEI affect the performance of a given cultivar may aid in selecting cultivars and their best environments for maximum yield. Through additive models of the ANOVA it is possible to describe the main effects of genotype and environment and determine whether GEI is a significant source of variation. However, it does not provide insights into the genotypes or environments that give rise to the interaction (Samonte et al., 2005). Regression approaches (Eberhart and Russell, 1966; Tai, 1971), variance component methods (Shukla, 1972), additive main effects and multiplicative Interaction (AMMI) analysis (Gauch and Zobel, 1997; Gauch et al., 2008; Gauch, 1992), yield stability statistic approaches (Kang, 1993) and GGE biplot analysis (Yan and Kang, 2003; Yan and Tinker, 2006; Yan, 2001) are major techniques in analyzing multi environmental trials. However, the AMMI model and GGE biplot analysis are between the most frequently used in recent years in part due to the graphical and visual analysis they offer particularly desirable when dealing with complex data structures and patterns (Gauch et al., 2008). AMMI, GGE and other SVD-based model families share some common features, but best practices require model diagnosis for each individual dataset to determine which member is most predictively accurate (Gauch, 2006). Here, GGE have been designed for conducting biplot analysis of research data (Yan et al., 2000, 2007). GGE stands for genotype main effect (G) plus genotype by environment interaction (GE), which is the only source of variation that is relevant to cultivar evaluation, allowing visual examination of the GEI pattern of MEYT data. Thus, mathematically, GGE is the genotype by environment data matrix after the environment means are subtracted. GGE analysis have been previously prove useful to identify and characterize disease resistance and yield stability of breeding material in field trials (Villegas-Fernández et al., 2009; Fernández-Aparicio et al., 2012; Rubiales et al., 2012; Flores et al., 2012, 2013) taking advance of the discrimination power vs. representativeness view of the GGE biplot effective in evaluating test environments.

In this work following recording of several agronomic and disease related traits and through GGE approaches we aimed to (1) determine the usefulness of the different locations for selection of superior oat genotypes with interesting agronomic traits and (2) identify oat germplasm with good adaptation to Mediterranean agroecological conditions, with special attention to the stability of yield and of resistance to crown rust across locations distributed over the Mediterranean Basin.

2. Materials and methods

2.1. Plant material and experimental design

An Oat Network consisting of 32 commercial varieties considered with potential under Southern Spanish conditions were supplied by the Andalusian Network of Agricultural Experimentation (RAEA) and were evaluated over four crop seasons at 6

contrasting locations along Mediterranean Basin, including field trials conducted in Spain, Egypt, Palestinian Territories and Tunisia. An environment was the combination of a year and location (Table 1). The cultivars studied were named in this study by the following numbers: (1) Acebeda, (2) Adamo, (3) Aintree, (4) Alcu-dia, (5) Anchuela, (6) Araceli, (7) Caleche, (8) Canelle, (9) Chambord, (10) Chapline, (11) Charming, (12) Condor, (13) Cory, (14) Edel-prinz, (15) Flega, (16) Fringante, (17) Fuwi, (18) Hamel, (19) Kankan, (20) Kantora, (21) Karmela, (22) Kassandra, (23) Kazmina, (24) Mirabel, (25) Mojacar, (26) Orblanche, (27) Pallidi, (28) Patones, (29) Prevision, (30) Primula, (31) Rapidena, and (32) Saia (Supplemental Table 2). Cultivars were developed by different institutions and/or companies (Supplemental Table 1) and released to the market in the last 20 years. Most of these cultivars were bred for the north-European agroclimatic conditions, since the oat crop has been traditionally considered as a cold adapted cereal crop. However, scare information is available about their field performance under the agroclimatic conditions of the Mediterranean area.

Palestinian trials were performed in a single-location (Tulkarm) over the growing seasons 2007–2008, 2008–2009 and 2009–2010 on a light clay chromic luvisol (FAO, 2011) experimental field; Egyptian trials were carried out in a single-location (Kafr El-Sheikh) over the growing seasons 2007–2008 and 2008–2009 on a loamy calcareic fluvisol (FAO, 2011) experimental field; Tunisian trial was done in a single-location (Beja), in the season 2007–2008 on a clay loam rendric leptosol (FAO, 2011) experimental field; Spanish trials were performed in three contrasting locations (Escacena with light clay eutric vertisol, Córdoba with light clay calcic cambisol and Salamanca with sandy loam or sandy-clay-loam Vertic Luvisol soils, respectively) during growing seasons 2009–2010 and 2010–2011 (Table 1). Sowings took place between October and December, according to local practices, except in Córdoba during the season 2009–2010 in which, due to intense rain levels, the sowing took place in January. No irrigation was performed in Palestinian, Spanish or Tunisian trials, but Egyptian plots were level basin flood irrigated according to local practise. This was done at sowing, and then on 1st of February and 1st of March by the application of 800 m³ ha⁻¹ each time. No artificial inoculation was performed at any location, infection occurring naturally. At each location, a randomized complete block design with three replicates was used. Each replicate consisted in independent plots consisting in 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 30 cm, at a sowing density of around 90 seeds m⁻². Hand weeding was carried out when required, and no herbicides or fertilizers were applied.

2.2. Disease, precocity, biomass and seed yield assessments

When disease symptoms were observed, disease severity (DS) was assessed as a visual estimation of the percentage of whole plant tissue covered by pustules of the crown rust. Observations were made weekly from disease onset until the end of the disease cycle. This allowed calculation of the area under the disease progress curve (AUDPC) according to Wilcoxson et al. (1975). Precocity was estimated as days to flowering by counting the number of days from sowing until 50% anthesis. At maturity stage, total above-ground dry matter was determined following field-drying of the plant material for at least 1 week. All grain was oven-dried at 70 °C. Yield are presented on an oven-dry basis of seeds weighted (kg/ha). Biomass data based on the above-ground plant weight (tones/ha) was taken for Spanish and Palestinian trials and harvest index (HI, %) was calculated as the ratio between grain weight to total dry matter.

Table 1
Description of the environments (combination of location and season) of the trials for the multi-environment study. Climatic data are provided for the growing season.

Environ	Location	Altitude (m ASL)	Pre-anthesis				Grain filling				Ripening				Total water (mm)	
			Time line	Average T _{max} (°C)	Average T _{min} (°C)	Rain (mm)	Irrigation (mm)	Time Line	Average T _{max} (°C)	Average T _{min} (°C)	Rain (mm)	Time line	Average T _{max} (°C)	Average T _{min} (°C)		Rain (mm)
Es09	Escacena, Spain	88	22-01/13-04	19.3	8.5	390.0	0.0	14-04/2-05	27.6	11.2	2.2	3-05/31-05	30.4	15.0	11.8	404.0
Es10	Escacena, Spain	88	17-12/12-04	18.2	7.2	307.3	0.0	13-04/5-05	26.5	13.1	49.8	6-05/30-05	34.1	15.9	3.2	360.3
Sa09	Salamanca, Spain	829	30-11/3-05	12.1	1.5	263.1	0.0	4-05/2-06	21.6	6.5	32.8	3-06/30-06	26.3	10.8	41.7	337.6
Sa10	Salamanca, Spain	829	1-11/27-04	13.2	1.6	186.9	0.0	28-04/20-05	24.6	8.8	22.9	21-05/15-06	28.2	10.3	22.9	232.7
Co09	Córdoba, Spain	90	2-12/18-04	16.1	6.3	846.0	0.0	19-04/30-04	26.7	13.8	4.2	1-05/30-05	27.6	27.6	32.7	882.9
Co10	Córdoba, Spain	90	30-01/25-04	17.0	6.6	520.6	0.0	26-04/22-05	25.2	13.1	135.6	23-05/10-06	32.0	32.0	14.5	670.7
Tu08	Beja, Tunisia	222	1-12/14-03	17.6	6.3	778.4	0.0	15-03/20-04	26.7	14.5	12.3	21-04/13-05	32.0	15.8	0.0	790.7
Eg07	Kafr El-Sheik, Egypt	8	1-12/31-03	21.8	3.9	88.3	240.0	1-04/25-04	28.8	9.4	0.0	26-04/22-05	32.6	14.9	0.0	328.3
Eg08	Kafr El-Sheik, Egypt	8	1-12/3-04	22.2	6.9	29.5	240.0	4-04/30-04	28.5	12.1	0.0	1-05/31-05	33.6	18.9	0.0	269.5
Pa07	Tulkarm, Palest.	75	15-11/28-02	19.3	10.8	436.0	0.0	1-03/20-04	25.5	11.5	11.5	21-04/15-05	29.5	18.0	2.0	449.5
Pa08	Tulkarm, Palest.	75	1-12/9-03	20.0	11.3	326.0	0.0	10-03/15-04	22.7	13.1	112.0	16-04/10-05	29.2	19.6	0.0	438.0
Pa09	Tulkarm, Palest.	75	30-11/6-03	20.9	13.3	366.0	0.0	7-03/10-04	24.9	14.8	42.0	11-04/15-05	29.8	19.9	4.0	421.0

2.3. Statistical analysis

2.3.1. Variance components and estimation of broad sense heritabilities

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_g^2) and error variance (σ_e^2) were estimated using Proc Varcomp of SAS® 9.3 (SAS Institute Inc.). Broad sense heritabilities were calculated as

$$H = 1 - \frac{\sigma_e^2}{b\sigma_g^2 + \sigma_e^2}$$

where b is the number of replicates. Arcsine transformations of the data which did not meet the conditions of normality and homogeneity (i.e. HI data), were performed to conform to the assumptions of ANOVA analysis.

Pearson correlations were calculated to detect statistical correlations between traits measurements. F -ratios used to test effects for randomized complete block experiments combining location-year environments were determined according to McIntosh (1983).

2.3.2. Heritability-adjusted genotype plus genotype × environment interaction analysis

GEE biplot analysis shows the factors (G and GE) that are important in genotype evaluation and that are also the sources of variation in GEI analysis of MEYTx data (Yan, 2001; Yan et al., 2000). Biplot analysis of genotype × environment interaction are particularly appropriate when using cultivars or breeding lines which after several cycles of selection may be reasonably considered as fixed (Yang et al., 2009). We here used the HA-GGE biplot (Yan and Holland, 2010) 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. The genotype by environment two-way table was first centred with the respective environment means, multiply by \sqrt{H} and then divided by the SD of the respective environment. The HA-GGE biplot shows the first 2 principal components (PC1 and PC2) derived from the previous two-way table of each trait to singular value decomposition (Yan, 2001; Yan et al., 2000). Singular value partitioning is achieved by providing a scaling factor f to obtain alternative cultivars and environment scores. We chose the most straight forward variant called symmetric scaling ($f=0.5$) since it bears most of the properties associated to other scaling methods (Yan, 2002). Analyses were made by a SAS® 9.3 (SAS Institute Inc.) programme for graphing GGE biplots developed by (Burgueño et al., 2003). 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. The polygon view of GGE biplot explicitly displays the which-won-where pattern (Yan, 2002). Thus, the polygon is formed by connecting the markers of the genotypes that are further away from the biplot origin such that all other genotypes are contained in the polygon. Genotypes located on the vertices of the polygon reveal the best or the poorest in one or other environment.

Data derived from biplots were tested statistically by non-parametric bootstrapping for constructing 95% confidence intervals on the basis of empirical distributions of estimated parameters. Because SVD needs to be done 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 (Yang et al., 1996).

3. Results and discussion

3.1. Oat agronomic trait means and estimation of broad-sense heritabilities

Data from Table 2 showed high differences on means and variances of the evaluated agronomic traits between the different tested environments. The environment Co10 and Pa07 showed the lowest and the highest yield and biomass values, respectively. Indeed in Co10 there was a high correlation between yield and biomass ($r=0.592$, $P<0.001$), but this correlation was not observed when all data for all environments were considered together ($r=0.333$, $P=0.06$) probably due to low correlation in the other environments. In relation with these two parameters, the harvest index (HI) also showed a high variation among environments. The HI is an important factor in crop production and its improvement has been one of the greatest achievements that differentiate commercial varieties from their wild antecessors (Gepts, 2004). The HI is the ratio of grain yield to total biomass and reflects partitioning of photosynthate between grain and harvestable product (Donald and Hamblin, 1976; Hay, 1995). Thus, a high HI means that the allocation of carbon is directed to grain instead to biomass production and a high HI can be considered as a good trait in breeding high-yielding cultivars. Correlation analysis (Table 3) showed a strong correlation between HI and grain yield ($r=0.71$, $P<0.001$), whereas no correlation was observed between HI and biomass. Flowering date ranged from 97 (Co10), to 175 (Sa10) days to anthesis. Early flowering was associated with relatively high temperatures and rain level whereas longer flowering dates were associated with low temperatures and rain during the pre-anthesis period (Tables 1 and 2). Overall, flowering date was negatively and significantly correlated with yield in the global analysis ($r=-0.5972$, $P<0.001$). This was expected since one of the mechanisms for drought resistance in Mediterranean areas is its avoidance by early completion of the life cycle. Rust severity was highly variable among locations and among years within a location. The highest severity was associated with high maximum temperatures during the day, moderate minimum temperatures during the night and moderate rain levels, which offer the best conditions for rust development. Early flowering was also correlated with low rust severity highlighting how these cultivars may be an advantage in the Mediterranean environments. As expected, the rust incidence was significantly and negatively correlated with yield (Table 3), highlighting the importance of rust resistance cultivars, to be available for a good oat crop performance in Mediterranean environments, as stated below with particular examples. Coefficient of variation for each environment was also high, near 20% for particular environments, indicating a very different response of the assessed cultivars to rust attack (Table 2). Overall, heritabilities were consistently high, especially for grain yield within environments pointing out to genetic variation as an important component of the inheritance of these traits.

Analysis of variance (Table 4) indicated significant differences ($P<0.001$) for genotype (G), environment (E) and genotype by environment (GE) interaction for all traits assessed. Explained variation by G accounted between 25.9% and 75.9% (yield and time to flowering, respectively) of the total variation (Table 4). The partitioning of G + GE interaction through HA-GGE biplot analysis showed that the first two principal components were significant factors for all traits, explaining 55–88% of total G + GE interaction (Table 4).

While the variance analysis can provide a general overview of variation and detect potential GE interactions, it generates no useful information about trends that may arise by interactions or create a viable method to select stable genotypes. Thus, GGE analysis which combines ANOVA and PC approaches to graphically display G and GE interactions to identify candidate genotypes with desirable and consistent performance across years and locations for each

trait was used. Several recent reviews have exhaustively compared and contrasted AMMI and GGE with respect to their suitability for GE analysis (Gauch, 2006; Yan and Tinker, 2006; Yan et al., 2007; Gauch et al., 2008). As highlighted by Gauch (2006) both share common features although differs in others, taking into account that best practices required model diagnosis, which should not be either over- or under-stated (Yan et al., 2007). In addition authors stress the need for use confidence regions in biplots to make critical decisions for genotype selection based on a statistical test (Yang et al., 2009). Here we used the HA-GGE biplots to infer both the utility of the environments in terms of selection response and the behaviour of the cultivars in the different environments (Yan and Holland, 2010). We used a rank-two approximation, that is, biplots were constructed using the scores derived from the first two PCs to approximate the information content of the two-way GE table. The first two PCs of the two-way GE table are the largest contributors to the total variability in the table. According to Yang et al. (2009) the first two PCs should account for approximately 60% of the (G + GE) variability and the combined (G + GE) effect should account for >10% of the (E + G + GE) variability before claiming the usefulness of biplots. These conditions were observed for all biplots. The lowest PC1 + PC2 sum was 55% for test environment of biomass (Fig. 1). However (G + GE)/(E + G + GE) was much higher than 10% (24%) in this biplot and more important the ME identification coincided with the other biplots including that performed for flowering date in which the first two PC counted for up to 88%. On the other hand we carried out bootstrapping of the data according to Yang et al. (2009), and constructed 95% confidence intervals for supporting the information derived from the biplots generated as stated in materials and methods.

3.2. Mega-environment identification

To identify and characterize potential GE interaction, different biplots were constructed. The environment-specific HA-GGE biplots for each trait shown in Fig. 1 was used to elucidate relationships among environments and to identify a potential mega-environment, defined as meaningful subset of similar environments (Yan and Tinker, 2006). The four biplots showed a very similar trend (also the one depicted for HI in Supplemental Fig. 1). Between 55% and 88% of the total variability was accounted for by the first two PCs. The cosine of the angle between two environmental vectors provided an estimate of their correlation coefficient. Although for all traits, environments were positively correlated (acute angles), a clear difference was observed between the environments comprising the locations of Egypt and Palestinian Territories and the rest of locations/environments. Furthermore, the lines perpendicular to the sides of the polygon (named with lower case Latin letters) that encloses all markers points (Fig. 1), divided the plot into sectors. Environments belonging to Egypt and Palestinian Territories fell in a different sector (i.e. c–d for yield, b–c for biomass, a–b for rust and HI biplots) than those from Spain and Tunisia. For flowering time (Fig. 1C) separation within sectors was not so clear but still differences between ‘fertile crescent environments’ and the rest was evident. This could be due to the relatively lower differences among environments for this trait which accounted the lower variation coefficient (overall only 3.50; Table 2). Judging from bootstrap confidence intervals for the two first PC’s environment scores of all the biplots, data showed no overlapping of the 95% confidence intervals between environments belonging to Egypt and Palestinian Territories and those from Spain and Tunisia (Supplemental Fig. 2). Therefore two significantly different mega-environments (ME) could be derived from the analysis, one comprising the locations of Córdoba, Escacena, Salamanca and Beja (ME1) and another comprising Kafr El-Sheik and Tulkarm (ME2). Climatologically, ME1 was characterized by

Table 2

Mean, standard error (SE), standard deviation (SD), coefficient of variation (CV) and broad-sense heritability (*H*) for yield (kg/ha), biomass (tonnes/ha), flowering time (days to anthesis), rust severity (AUDPC rust) for each environment and mega-environment (ME) classification and mean within each mega-environment (Mean ME) for each trait.

Trait	Environment	Mean	SE	SD	CV	<i>H</i>	ME	Mean ME	
Yield (kg/ha)	Es09	686	167.6	465.7	18.4	0.96	1	947,8	
	Es10	1215	221.2	565.9	18.2	0.95			
	Sa09	1334	271.5	564.2	17.3	0.92			
	Sa10	1349	372.1	822.9	17.6	0.93			
	Co09	938	163.1	357.4	17.4	0.93			
	Co10	490	100.3	207.2	18.5	0.92			
	Tu08	622	113.6	353.1	18.3	0.97			
	Eg07	1594	190.7	618.5	12.0	0.97	2	3322,1	
	Eg08	1811	185.3	434.8	10.2	0.94			
	Pa07	6092	829.9	1840.1	13.6	0.93			
	Pa08	2899	411.1	1628.4	14.2	0.98			
	Pa09	4215	554.9	954.4	13.2	0.89			
	Es09	3235	357.4	1161.5	11.1	0.97			
	Es10	5273	467.1	1359.4	8.9	0.96			
Biomass (tonnes/ha)	Sa09	3128	415.4	1090.5	13.3	0.95	1	4098,2	
	Sa10	4991	646.7	1984.9	12.9	0.97			
	Co09	5228	606.1	1374.5	11.6	0.94			
	Co10	2734	354.6	746.9	12.9	0.93			
	Pa07	17170	815.3	5354.2	4.7	0.99			
	Pa08	10754	382.6	3897.3	3.6	0.99	2	13048	
	Pa09	11320	698.4	2948.9	6.2	0.98			
	HI	Es09	0.20	0.08	0.08	17.7	0.81	1	0,19
		Es10	0.18	0.08	0.04	18.4	0.37		
		Sa09	0.15	0.05	0.04	11.4	0.82		
Sa10		0.14	0.07	0.04	17.6	0.72			
Co09		0.28	0.06	0.03	11.7	0.11			
Co10		0.19	0.07	0.05	16.1	0.54	2	0,28	
Pa07		0.26	0.08	0.07	16.2	0.70			
Pa08		0.30	0.04	0.10	14.2	0.57			
Pa09		0.28	0.08	0.06	15.2	0.48			
Es09		167	5.6	4.2	3.4	0.57			1
Es10	136	5.4	10.8	4.0	0.92				
Sa09	171	7.6	5.9	4.4	0.47				
Sa10	176	7.4	11.1	4.2	0.85				
Co09	157	3.9	9.4	2.5	0.94				
Co10	97	4.5	12.0	4.7	0.95				
Tu08	151	5.1	9.2	3.4	0.90				
Eg07	143	2.6	11.5	1.8	0.98				
Eg08	143	2.5	11.6	1.7	0.99				
Pa07	147	4.1	14.5	2.8	0.97	2	138,4		
Pa08	131	5.8	13.4	4.4	0.94				
Pa09	128	5.7	13.6	4.5	0.94				
AUDPC rust	Es09	6.63	0.9	4.0	13.5	0.98	1	2,6	
	Es10	2.16	0.7	2.4	19.4	0.98			
	Sa09	0.75	0.2	0.6	19.2	0.96			
	Sa10	0.88	0.5	1.7	13.4	0.97			
	Co09	0.43	0.2	0.5	19.4	0.97			
	Co10	3.70	0.7	2.4	18.1	0.97			
	Tu08	3.43	0.7	2.4	18.3	0.97			
	Eg07	1.03	0.5	1.2	16.7	0.94	2	0,9	
	Eg08	1.24	0.3	1.2	17.6	0.97			
	Pa07	0.15	0.1	0.3	17.2	0.96			
	Pa08	0.94	0.2	0.9	15.1	0.98			
	Pa09	0.95	0.2	0.5	19.6	0.94			

higher altitude, lower maximum and minimum temperatures and higher rain level than ME2 during both anthesis and grain filling period. ME2 produced the highest grain yield and biomass and was also characterized by a shorter flowering time and lower rust incidence (Table 2). The high correlation between yield and biomass ($r=0.592$, $P<0.001$) observed overall for ME1 (Table 3) was not observed when data for all environments were considered ($r=0.333$, $P=0.06$) due to the low correlation found in ME2 (Table 3). Interestingly, a significant positive correlation was found between HI and biomass in ME1 whereas this correlation was negative for ME2 leading to the no correlation observed when considered all data (Table 3). These differences might be explained taking into account the flowering data. Flowering date was negatively and significantly correlated with yield ($r=-0.6563$, $P<0.001$)

in ME2. This high influence of flowering time over yield particularly in the hotter and drier locations might be related with the ability of early flowering cultivars to complete its cycle before appearance of severe drought. Thus, in the hotter and drier ME2 locations long life cycles would be correlated with low yield, probably due to the extreme climatic conditions during grain filling. However, in ME1 with moderate temperatures and rain level during grain filling period, long cycles might contribute positively to biomass and grain yield.

To conduct test environment evaluation, it is essential to first conduct a mega-environment analysis, that is, to investigate whether Mediterranean Basin oat growing region can be divided into mega-environments, because test environment evaluation as well as genotype evaluation becomes meaningful only when

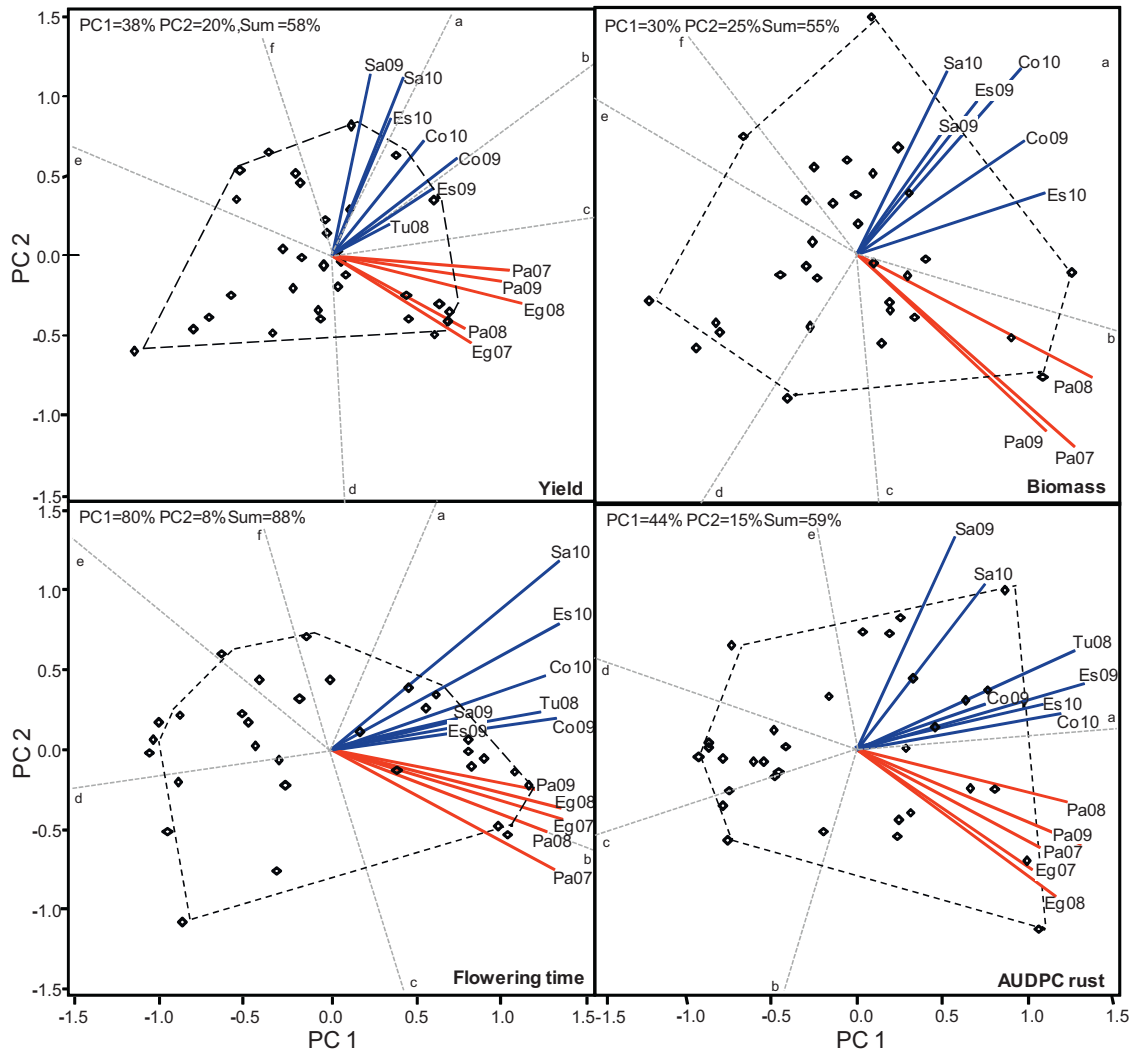


Fig. 1. Heritability-adjusted genotype plus genotype × environment interaction (HA-GGE) biplot for mega-environment identification for Spain, Tunisia, Palestinian Territories and Egypt between 2007 and 2011. The traits assessed were (A) Yield, (B) Biomass, (C) Flowering date, (D) Rust incidence.

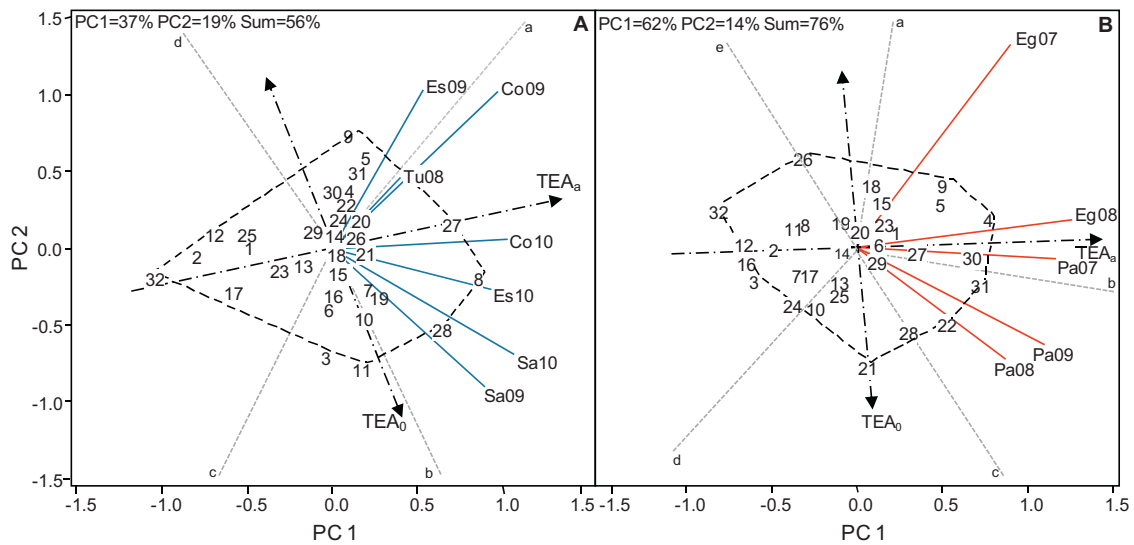


Fig. 2. HA-GGE biplot based on the grain yield (kg/ha) of 32 oat cultivars grown at 12 location-year environments, from 2007 to 2010. (A) ME1 constituted by Spanish and Tunisian locations, (B) ME2 constituted by Egyptian and Palestinian locations. PC, principal component.

Table 3
Correlation matrix of evaluated agronomic traits.

	Rust	Yield	Floración	Biomass
<i>Global</i>				
Yield	-0.4093*			
Flowering	0.5584***	-0.5972***		
Biomass	0.0807	0.3334	0.0348	
HI	-0.3840*	0.7107***	-0.5307**	-0.0592
<i>ME1</i>				
Yield	-0.2677			
Flowering	0.5432***	-0.1956		
Biomass	-0.3502*	0.6392***	-0.3284	
HI	-0.2868	0.8083***	-0.3866*	0.5678***
<i>ME2</i>				
Yield	-0.4687**			
Flowering	0.5190**	-0.6563***		
Biomass	0.0346	0.1708	0.1523	
HI	-0.4054*	0.3672*	0.5225*	-0.4234*

* Significant at the 0.05 level of probability.
 ** Significant at the 0.01 level of probability.
 *** Significant at the 0.001 level of probability.

Table 4
Genotype (G), location-year environment (E) and genotype by location-year environment interaction (GE) terms for biomass, grain yield, days to flowering, AUDPC rust and AUDPC mildew for the spring oat performance trials, from 2007 to 2010.

Trait	Source of variation	df ^a	Mean squares	Explained variation % of G,	% of PC1 + PC2 ^c	G + GE/(E + G + GE)
Grain yield	E	11	271600000***			
	G	31	7261757***	25.9	38 + 20	0.23
	GE	341	1888374***			
Biomass	E	8	2.323 10 ⁹ ***			
	G	31	4.96 10 ⁷ ***	26.13	30 + 25	0.24
	GE	248	1.753 10 ⁷ ***			
HI	E	8	0.51121***			
	G	31	0.05409***	32.27	47 + 15	0.56
	GE	248	0.01419***			
Flowering	E	11	45010.2***			
	G	31	3304.8***	75.9	80 + 8	0.21
	GE	341	25.9***			
AUDPC rust	E	4	337.269***			
	G	31	42.862***	34.86	50 + 15	0.51
	GE	124	0.242***			

^a Degrees of freedom.
^b Percentage sums of squares respect from the total sums of squares.
^c Proportions of the first two principal components derived from singular value decomposition of the HA-GGE analysis.
 *** Significant at the 0.001 level of probability.

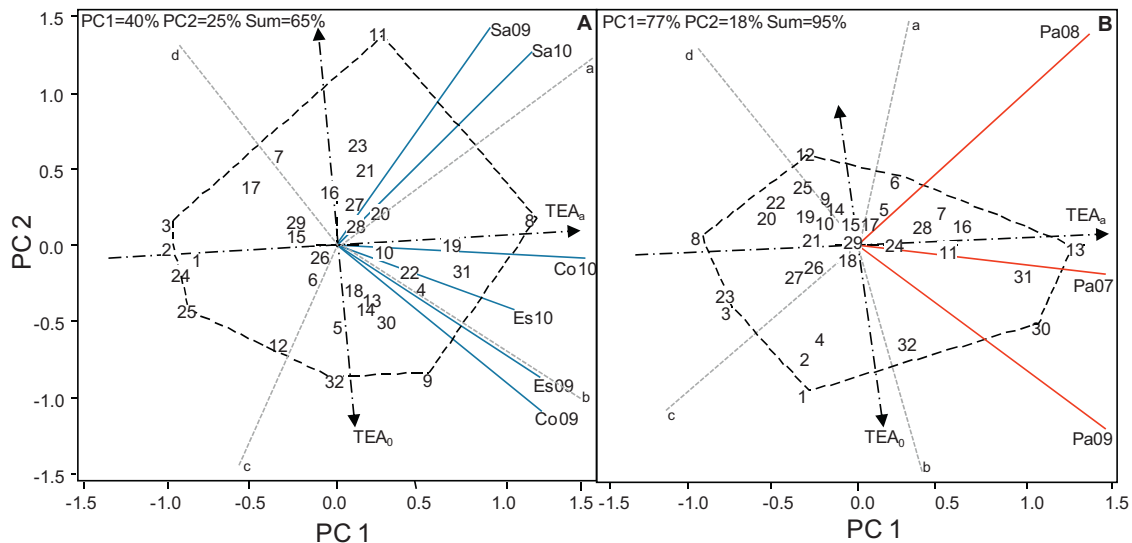


Fig. 3. HA-GGE biplot based on biomass (Tones/Ha) of 32 oat cultivars grown at 9 location-year environments, from 2007 to 2010. (A) ME1 constituted by Spanish and Tunisian locations, (B) ME2 constituted by Egyptian and Palestinian locations. PC, principal component.

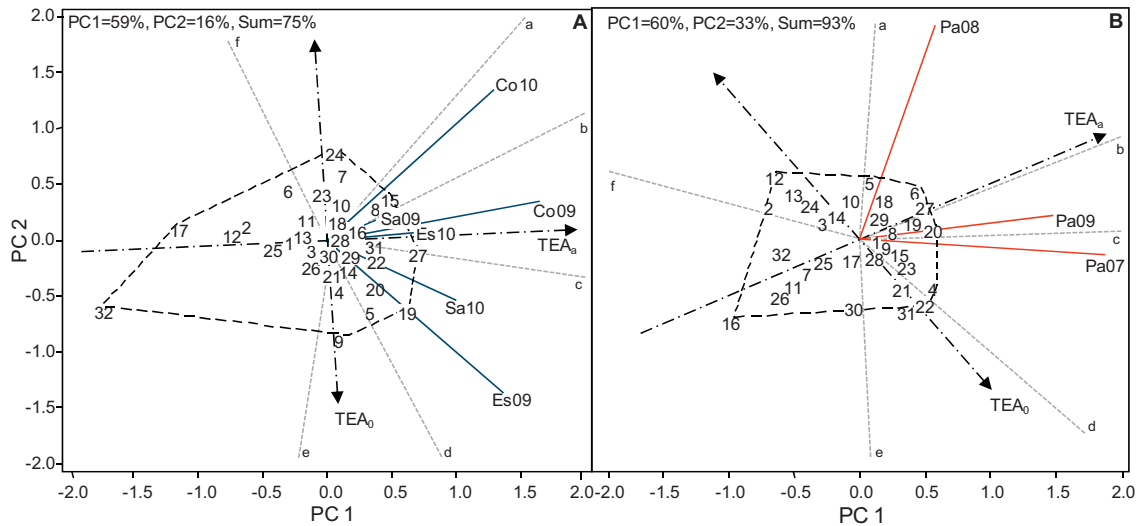


Fig. 4. HA-GGE biplot based on HI of 32 oat cultivars grown at 12 location-year environments, from 2007 to 2010. (A) ME1 constituted by Spanish and Tunisian locations, (B) ME2 constituted by Egyptian and Palestinian locations. PC, principal component.

conducted within mega-environments (Yan et al., 2007). According to our results, for further analysis of the specific traits, two biplots, one per ME, were developed in order to assess the usefulness of environments for selection and the best performing genotypes (Figs. 2–6).

3.3. Test environment evaluation

One of the crucial factors for the success of a plant breeding programme is to identify suitable breeding and testing locations. For a location to be suitable, it must be discriminating so that the genetic differences among genotypes can be easily observed, representative of the average environment so the selected genotypes have the desired adaptation and repeatable so that selected genotypes during one year will have good performance in the coming years. According to Yan and Holland (2010) and Yan et al. (2011) in the HA-GGE biplots, the vector length of an environment will be proportional to the square root of the heritability in the environment (\sqrt{H}) and therefore indicative of its discrimination power;

the cosine of the angle of between an environment with TEA_a (average environment) or between two environments indicates the genetic correlation (r) between them and is an indicative of the representativeness and repeatability, respectively and the projection of the vector onto the TEA_a should approximate $r\sqrt{H}$ which is an overall measure of the usefulness of an environment (Allen et al., 1978; Flores et al., 2013). In the HA-GGE biplots derived in this work, approximately 56–88% and 75–97% of the total variability was accounted for by the first two PCs for ME1 and ME2, respectively and in all of them the combined (G + GE) effect accounted for more than 10% of the (E + G + GE) variability indicating the adequacy of the rank-two approximation.

Focusing in Grain Yield (Fig. 2A and B), Córdoba together with Escacena showed as the most useful location for selecting superior yielding genotypes within the ME1 according to biplots (Fig. 2) with long projections over the TEA_a , long vectors and small angles (approx. 30° or smaller) with the average axis indicating their usefulness for genotype discrimination and a high representativeness of the ME (Fig. 2A). The small angle between years (slightly higher

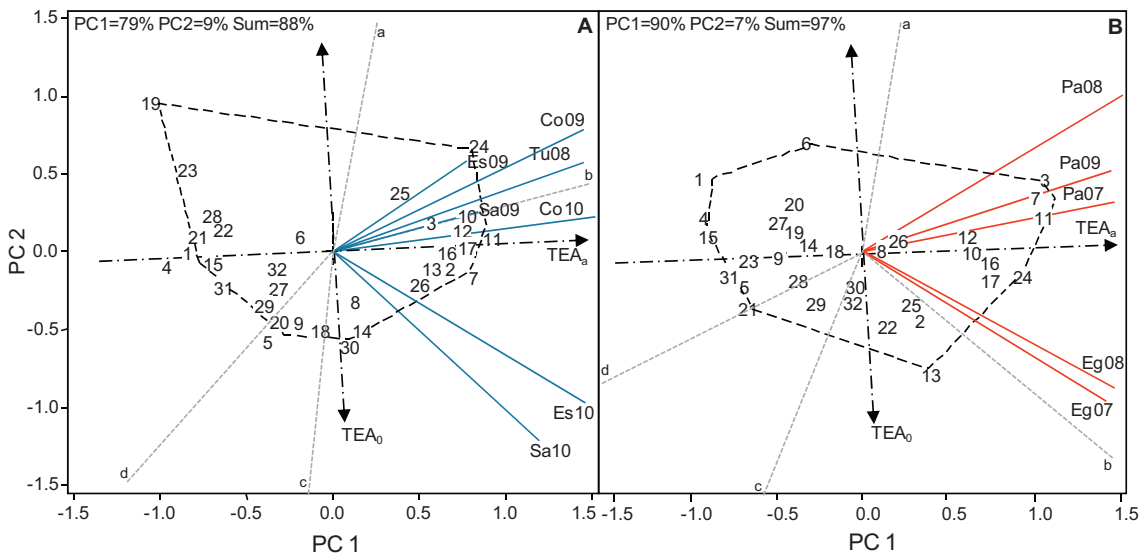


Fig. 5. HA-GGE biplot based on days to flowering of 32 oat cultivars grown at 12 location-year environments, from 2007 to 2010. (A) ME1 constituted by Spanish and Tunisian locations, (B) ME2 constituted by Egyptian and Palestinian locations. PC, principal component.

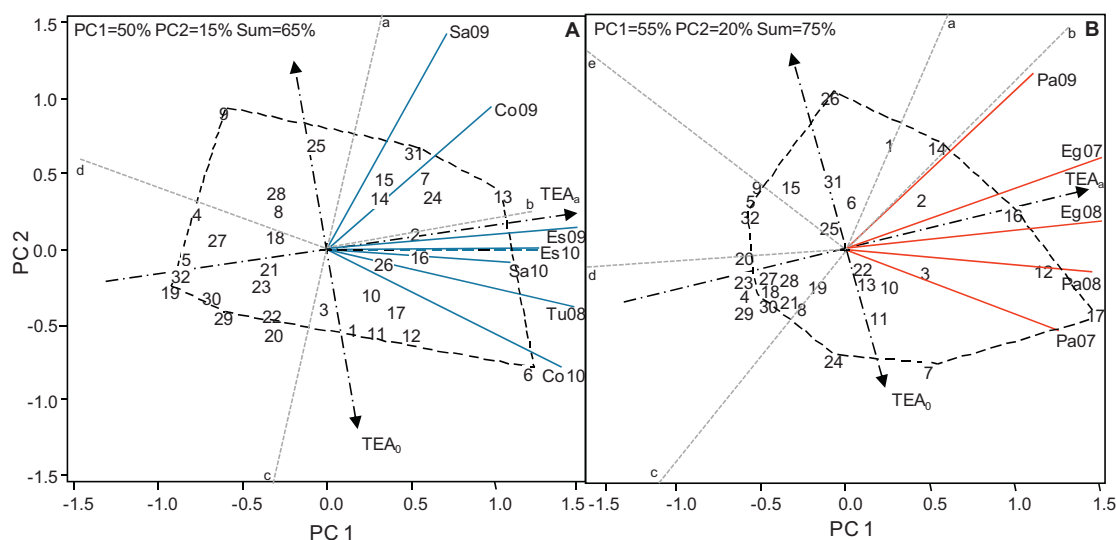


Fig. 6. HA-GGE biplot based on rust incidence on 32 oat cultivars grown at 12 location-year environments, from 2007 to 2010. (A) ME1 constituted by Spanish and Tunisian locations, (B) ME2 constituted by Egyptian and Palestinian locations. PC, principal component.

than 30°) in Córdoba location reflected its repeatability, more than for Escacena, and therefore, Córdoba could be considered as a Type I location according Yan et al. (2011), ideal for selecting superior genotypes within ME1. Indeed the 95% confidence interval graphs showed no significant differences between Co09 and Co10 whereas it discriminated between Es09 and Es10. The two environments corresponding to Salamanca location, Sa09 and Sa10, although showed an acute angle between both and therefore a high repeatability supporting by overlapping of the 95% confidence intervals for this two environments, their projections on the TEA_a axis were small should not be used for selecting high yielding genotypes for ME1 since the high angles with the average environment (higher than 45°) indicate that they were not highly representative of this ME (Fig. 2A). Indeed the 95% confidence interval graph showed significant differences between this location and the rest of environments of ME1 albeit not as higher as those observed between environments corresponding to the 2 MEs. According to biplots (Fig. 2B), Palestinian location seemed appropriate for grain yield selection in ME2, since although Eg08 showed a high projection over the TEA_a, Eg07 showed a high angle with the average axis and therefore low representativeness for this ME. However the 95% confidence intervals showed no clear differences between Palestinian and Egyptian locations.

Regarding biomass production (Fig. 3A and B), with exception of Es10 all environments showed as useful locations for biomass selection in ME1 (Fig. 3A), with long projections on the TEA_a axis. This data were supported by the 95% confidence interval graphs that pointed out Es10 as significantly different from the others. Biomass was only assessed at the Palestinian location and it was useful for biomass selection in ME2 with angles under 45° with respect to the average environment (representativeness) and long vector (useful for discriminating among genotypes) (Fig. 3B).

Concerning the HI (Fig. 4A and B), Córdoba location showed the highest projections on the TEA_a axis pointing out the usefulness of this location within the ME1 (Fig. 4A). This location showed a high discriminating power, reflected by the vector length of both seasons, and a high repeatability as shown by the acute angle between both years. HI was only assessed at the Palestinian location in ME2 and showed angles under 45° with respect to the average environment (good representativeness), long vectors (useful for discriminating among cultivars) and moderate to high projection on the TEA_a axis (moderate to high usefulness), suggesting that

Palestinian location were adequate for the evaluation of this trait (Fig. 4B).

With respect to flowering time (Fig. 5A and B), Córdoba location showed the highest projections on the TEA_a axis together with Beja (Tunisia) indicating the usefulness of these locations. Indeed, the 95% confidence interval graph showed no significant differences between these two locations (Supplemental Fig. 2). In addition, the high repeatability observed at Córdoba according to the acute angle between vectors and their proximity in the confidence interval graph, together with the high representativeness given by the small cosine of the angle of between each environment with TEA_a, led us to consider Córdoba between the most appropriate locations for selecting cultivars according to the flowering date. As previously observed with yield and biomass, Salamanca location showed lower usefulness for cultivar selection according to flowering date. Indeed, although Sa09 was similar to Es09, Sa10, significantly different from the other environments (Supplemental Fig. 2) showed a relatively high angle with the TEA_a indicating a low representativeness of ME1 (Fig. 5A). By contrary both locations assessed for ME2 (Fig. 5B) showed high projections over the TEA_a axis and therefore could be considered useful for cultivar selection since no significant differences between locations were inferred from the 95% confidence intervals analysis.

According to biplot analysis, Escacena location (Es09 and Es10), with very high projection over TEA_a showed as the most appropriate environment for selecting rust resistant cultivars in ME1. Indeed, it showed quite small angles with the average environment (smaller than 20°) indicating a high representativeness, long vectors indicating the usefulness for discriminating between resistant and susceptible cultivars (Fig. 6A) and small angles between both seasons reflecting its high repeatability. However confidence interval graph showed no differences between this location and Córdoba, indicating also the usefulness of this location with long projections and relatively small angles with TEA for selecting rust resistant cultivars. In ME2 (Fig. 6B), both Egyptian and Palestinian environments showed high projections on the TEA_a axis, indicating they were useful locations for selecting rust resistant cultivars, indeed the confidence interval graph showed no differences between most of the environments comprising ME2.

In general for ME1, Córdoba showed good characteristics for cultivar selection for yield, biomass and flowering date with long vectors (indicative of discrimination power), small angles

(indicative of representativeness within the ME), high projections on the TEA_a (indicative of the usefulness) and a moderate repeatability between seasons. Salamanca showed in most biplots as the lowest representative location for ME1. This could be attributed to the low maximum and minimum temperatures associated with the high altitude of this location. For ME2 these big differences were not observed between the two locations studied and for most traits, both locations showed no significant differences.

3.4. Genotype evaluation

Genotype evaluation was carried out for each specific ME. An ideal cultivar should have both high mean performance and high stability within a ME. These characteristics may be inferred from the projection of each cultivar over the TEA_a, which indicates the mean performance for a specific trait across all environments and over the TEA₀, which indicates the stability (Yan, 1999). When the different environments within a ME fall in different sectors, the vertex cultivar indicates the won cultivar for each specific environment (Yan et al., 2007).

3.5. Grain yield

On ME1, the HA-GGE biplot for grain yield (Fig. 2A) shows a six-sided polygon formed by the union of the vertex cultivars 9, 27, 8, 28, 11 and 32. Of these, 8, 28 and 27 were the highest yielding cultivars significantly different from the others according to the bootstraps and 27 was also the most stable cultivar across over the environments tested. Indeed cultivar 27 was among the 10% of the highest yielding cultivars in 4 out of 7 environments according to the raw means (i.e. 2140 kg ha⁻¹ in Sa09). In contrast, cultivars 32, 2, 12 and 17 yielded significantly poorly at all environments. These data are supported by the raw means that showed cultivar 32 as the lowest yielding in 6 out of 7 environments tested (i.e. 172 kg ha⁻¹ in Co09). As expected, grain yield was strongly affected by agroclimatic conditions. A detailed look at Table 1 can help us to understand these results. We did not observed big differences between average maximum temperature and average minimum temperatures for all Spanish environments between both seasons (Table 1). However, we observed differences in the precipitation levels. Higher rainfall levels recorded in Escacena during the grain filling period in the season 2010–2011, could help in the increase of yield observed respect to the previous season. In Córdoba 2009–2010, and excess of rainfall concentrated particularly during the month following the sowing lead to a worse crop establishment which could be the cause of the lower yield observed in this season. Cultivar 9 and 5 could be considered as high yielding cultivars under low rainfall conditions during grain filling period whereas cultivars 8 and 28 were considered high yielding grain cultivars in those environments with moderate rainfall levels during the grain filling period (Fig. 2A).

In ME2 (Fig. 2B), the highest yielding cultivars were 4, 30 and 31, significantly different from the rest of cultivars. Indeed they were among the 10% of the highest yielding cultivars according to raw means in 4 out of the 5 environments tested. According to biplots the most stable cultivar was 30 although it was not significantly different from 4 and 31. Cultivars 9 and 5 were unstable for seed yield as it performed well at Eg08 and Eg07 environments but moderately at the rest of environments. By contrast, 22 and 28 were moderately high yielding cultivars performing better in Palestinian than Egyptian locations. On the other hand, cultivars 12 and 16 yielded poorly at all environments in a stable way together with cultivars 32 and 3 that showed higher instability across all environments. Indeed, cultivar 16 showed among the lowest yields in 4 out of the 5 environments tested (i.e. 937 kg ha⁻¹ in Eg07).

Interestingly, two cultivars (9 and 5) with high grain yield under low rainfall levels during grain filling in ME1 (Fig. 2A), exhibited good grain yield performance in Egypt during both seasons. By the contrary, 31, 22 and to a lesser extent 28, showed good yield in the Palestinian location, where moderate levels of precipitation were recorded during the three seasons confirming the trend observed for ME1. Furthermore, cultivar 12, 2 and 32 which were among the poorest yielding cultivars in ME2 showed also a poor (moderately unstable) grain yield in ME1, too. Thus, we could consider these cultivars as the poorest grain yielding across all environments tested in the Mediterranean Basin.

3.6. Plant biomass

There is general agreement that, in suitable climates, oats are well suited to haymaking. Oats ensile well for on-farm use. Oat straw is a valuable feed resource, more palatable to stock and more nutritious than the straw of wheat or barley. However, few breeding programmes are engaged in fodder oats, so the common situation is that cultivars are bred and grown mainly for grain, and the same cultivars are used for grain as for forage (Kirilov, 2004).

The HA-GGE biplot for biomass (Fig. 3A and B) showed the behaviour of the different cultivars respect to biomass production in the two differentiated ME. In ME1, Fig. 3A, showed a seven-sided polygon formed by the vertex cultivars 11, 8, 9, 32, 25, 24, 2 and 3, which were the most extreme cultivars for each of the sectors formed. Cultivar 8 was considered the highest biomass producing cultivar with the longest positive projection on the average environmental axis TEA_a and the highest stability defined by its projection on TEA₀ close to zero (Yan, 1999). It was significantly different from any other cultivar according to the bootstrap analysis and was within the 10% of the highest yielding according to the biomass raw yield in 3 out of the six environments tested. Interestingly this cultivar was also among the highest grain yielding cultivars, indicating its suitability for both, grain and forage. Cultivars 19 and 31 showed also a good biomass and an acceptable stability over environments (Fig. 3A). Indeed 13 out of the 18 cultivars that showed positive projection over TEA_a in the yield biplot for ME1 also showed positive projections in the biomass biplot. This correlation between yield and biomass derived from biplots confirmed the previous results from Pearson correlation (Table 3). On the other hand, cultivar 11 did not show a good behaviour in terms of biomass production when considering the target ME1 (average environment). However it was the best for both biomass production and also grain yield in the colder location of Salamanca being also within the best 10% cultivars in both environments of this location according to the raw means. This suggests a better adaptation of this cultivar under lower temperature requirements. On the other side of the biplot, we found cultivars, 3, 2, 1, 24, and 25, which according to the 95% confidence intervals were not significantly different among them, showing the lowest biomass and stable with respect to the target environment. From these, cultivar 2 also showed a very poor yield in ME1 (Fig. 2A).

In ME2, cultivar 13 showed significantly ($P < 0.05$) the highest biomass production (positive projection on TEA_a) and the highest stability (projection on TEA₀ close to zero). It was the winner cultivar in Pa07 and Pa08 and with the bets 10% in Pa09 according to the raw means. Cultivars 30 and 31 (not significantly different between them) also showed a high biomass production (Fig. 3B) and although they showed a moderate stability over environments they showed also a high yield in ME2 (Fig. 2B), hence they would be suitable for both, yield and forage. On the opposite side of the plot, cultivar 8 was significantly the lower biomass producing cultivar respect to the target environment and was also a low yielding cultivar (Fig. 2B). However this cultivar was one of the highest yield and biomass producing for ME1 suggesting that it was most adapted to

moderate maximum and minimum temperatures and had higher rain level requirement for optimum performance, mainly during grain filling period.

3.7. Harvest index

Fig. 4A and B illustrates the HA-GGE biplot for the harvest index. On ME1, (Fig. 4A), cultivar 27 showed the highest HI (positive projection on TEA_a) and the highest stability (projection onto TEA₀ closed to zero) although according to bootstrap analysis it was not significantly different from cultivars 31, and 22. In the opposite side of the plot, we found cultivar 17 with a very low HI (negative projection on TEA_a) displaying moderately stability across all the environments tested (moderate projection on AEC₀) and cultivar 32 also with a very low HI but more instability. Both cultivars, 17 and 32 showed a significantly different behaviour between them and respect to the other cultivars. As expected according to the correlations performed (Table 3) most of the cultivars with positive projection in the yield and biomass biplots (Figs. 2A and 3A) also showed a positive projection in the HI biplot (Fig. 4A). Interestingly in ME2 (Fig. 4B) cultivar 27 were among those with the highest HI and stability.

3.8. Days to flowering

As previously stated, the flowering date of the different cultivars was the assessed parameter that showed less variation between the two MEs. Indeed cultivars 7, 11, 24 and 3 from one side and cultivars 4, 1, 21, 15 and 31 on the other side were significantly among those with the longest and shortest flowering time in both ME, respectively (Fig. 5A and B). This data were supported by the raw means that followed a similar trend. Interestingly, in ME2 those cultivars with short flowering time such as 31 and 4 were among the high yielding cultivars (Fig. 2B), supporting the suggestion that for this ME characterized for higher temperatures and low rain levels particularly in the grain filling period a short cycle may constitute a way to escape for drought allowing a better performance. This trend was not observed in ME1 with lower temperatures and higher rain levels.

3.9. Rust infection

HA-biplots for rust (area under the disease progress curve; Fig. 6A and B) confirmed the previous correlations indicating a negative correlation between rust and yield particularly in ME2. In this ME cultivars such as 16, 12 and 17 that significantly differed from the others and had the highest rust infection and the lowest yield. Since the highest AUDPCs were observed in locations from ME1, such as Córdoba and Escacena the explanation of the higher influence of rust disease on yield in ME2 may arise from the growth stage at which infections were observed. Indeed, rust infection appeared associated to high temperatures in ME1 and coincided with the last part of the pre-anthesis period and grain filling, whereas in ME2 coincided with late stages of grain filling period. Interestingly, resistance response of cultivars against rust in ME1 was significantly ($P < 0.001$) associated with the response in ME2 ($r = 0.633$). This might indicate that similar or related isolates were the responsible of the disease at both ME and/or that resistance responses based on non-hypersensitive mechanisms were particularly important in the final response. Supporting the latter, in a previous work under controlled conditions several of these cultivars were histologically assessed to determine the mechanisms underlying the resistance response against to the rust isolate from Córdoba (Sánchez-Martín et al., 2012). Particularly those that showed the lowest disease in field such as cultivars 19, 32, 5, 30 and 4 (Fig. 6A) were characterized by a high level of pre-penetration resistance and also by

a high percentage of early aborted colonies not associated with host cell necrosis (Sánchez-Martín et al., 2012). Prehaustorial resistance plays a major role in so-called partial resistance. It is a wide spectrum resistance based on multiple and quantitative genes, and therefore it is more difficult to overcome by new races of pathogens than other resistance mechanisms based on single or qualitative genes, such as those promoting cell death (Niks and Rubiales, 2002). In addition, some of them such as cultivar 19, 30 and 4 showed also high levels of hypersensitive response leading to a very low disease severity. The knowledge of the productive and disease resistance features of the cultivars here evaluated may ease the grower's decision on cultivar selection. Thus, if their area have traditionally a high incidence of the rust disease at the moment of grain filling (i.e. ME2) the loss of productivity of cultivar 4 with respect for instance cultivar 28 will be compensated with their disease resistance achieving a higher final yield (Fig. 2B) whereas in ME1 it may be better the selection of cultivar 28 (Fig. 2A).

4. Conclusions

Availability of cultivars with good yield potential and resistance to major biotic and abiotic stresses is needed for sustainable oat crop production. In this work we studied the adaptation of a series of oat varieties to a drought prone area such as the Mediterranean Basin by assessing agronomic traits and further studied the stability of the resistance to rust incidence across several crops seasons and countries. Each trait analyzed separately, showed different responses of the accessions. However, as the variations in ranking show, it is necessary to exam the stability of the material and its interaction with the environment. ANOVA analysis demonstrated that the environments were different and contrasting, with important interaction with genotype. All of this justifies the uses of HA-GGE biplot method to analyze the data from multi-environment trials (Crossa and Cornelius, 1997). Biplots revealed two clear different ME, one in the “crescent fertile” and another grouping the rest of locations. This study also allowed the discrimination of the best and worst cultivars for the different evaluated traits within each ME. Overall, this work will ease the breeding for oat adaptation within the Mediterranean area.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2013.10.018>.

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