

## Progress in the Spanish National Barley Breeding Program

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### Abstract

The Spanish Barley Breeding Program is carried out by four public research organizations, located at the most representative barley growing regions of Spain. The aim of this study is to evaluate the program retrospectively, attending to: i) the progress achieved in grain yield, and ii) the extent and impact of genotype-by-environment interaction of grain yield. Grain yields and flowering dates of 349 advanced lines in generations F8, F9 and F10, plus checks, tested at 163 trials over 11 years were analyzed. The locations are in the provinces of Albacete, Lleida, Valladolid and Zaragoza. The data are highly unbalanced because the lines stayed at the program for a maximum of three years. Progress was estimated using relative grain yield and mixed models (REML) to homogenize the results among years and locations. There was evident progress in the program over the period studied, with increasing relative yields in each generation, and with advanced lines surpassing the checks in the last two generations, although the rate of progress was uneven across locations. The genetic gain was greater from F8 to F9 than from F9 to F10. The largest non-purely environmental component of variance was genotype-by-location-by-year, meaning that the genotype-by-location pattern was highly unpredictable. The relationship between yield and flowering time overall was weak in the locations under study at this advanced stage of the program. The program can be continued with the same structure, although measures should be taken to explore the causes of slower progress at certain locations.

**Additional key words:** genotype-by-environment interaction; *Hordeum vulgare*; pedigree selection.

### Resumen

#### Progreso en el programa nacional español de mejora de cebada

El programa nacional de mejora de cebada se lleva a cabo por cuatro organismos públicos situados en las principales regiones productoras de cebada de España. El objetivo de este estudio es la evaluación retrospectiva de i) el progreso en términos de rendimiento y ii) la magnitud y el efecto de la interacción genotipo-por ambiente del rendimiento en los materiales avanzados. La base de datos utilizada consiste en datos de rendimiento absoluto y relativo y fechas de floración de 349 líneas F8, F9 y F10, además de testigos, evaluadas en 163 ensayos distribuidos en 11 años. Las localidades de ensayo están en las provincias de Albacete, Lleida, Valladolid y Zaragoza. El progreso del programa se estimó utilizando el rendimiento relativo analizado mediante modelos mixtos para homogeneizar los resultados entre años y localidades, que son muy desequilibrados. Se constató la existencia de progreso en el programa, aumentando los rendimientos en cada generación, hasta superar a los testigos, aunque el progreso varió entre provincias y entre localidades. La ganancia genética fue mayor de F8 a F9 que de F9 a F10. El componente de varianza más grande (además de los puramente ambientales) fue el de genotipo por localidad y por año, por lo que los patrones geográficos eran imprevisibles. La relación entre fecha de floración y rendimiento en general fue débil en todas las localidades en esta etapa avanzada del programa. El programa puede continuar con la misma estructura, pero se debería investigar la causa del menor progreso obtenido en algunas localidades.

**Palabras clave adicionales:** *Hordeum vulgare*; interacción genotipo por ambiente; selección genealógica.

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## Introduction

Barley, *Hordeum vulgare* L., is one of the most important cereal crops in the world (Baik & Ullrich, 2008), and it is grown in regions with climates unfavorable for production of other major cereals. It is commonly grown under dry conditions, poor and even saline soils, where it has a productive advantage. Because of these characteristics, it has been the principal grain produced in numerous stress-prone areas (Pohlman, 1985; Guttier *et al.*, 2001), including the Mediterranean basin. In 2009, the barley cultivation area in Spain was 3.05 million hectares, and the production was 7.35 million tons, which corresponded to 22% of the total area devoted to barley in the European Union, and 13.5% of the total production (FAOSTAT, 2011). It is the first crop in terms of acreage in Spain, being mostly grown in dry inland areas.

Despite being such an important crop for Spain, the breeding activities carried out by private companies are almost non-existent. The reason is the low profit obtained from sales of seed, as less than 10% of the surface is sown to certified seed. As a consequence, most cultivars available to growers in Spain have been bred in other countries. Even though some of these cultivars perform quite well in Spain, we expect that local breeding should result in superior cultivars. Studies carried out in the Mediterranean region have demonstrated that the most effective way to improve productivity of crops grown in less-favored areas is to use locally adapted germplasm and select in the target environment(s) (Ceccarelli, 1994; Ceccarelli *et al.*, 1998). The Spanish program takes advantage of this approach by local testing and also by the use of local landraces (Lasa, 2008) as source of adaptation traits.

Therefore, there was a need to provide Spanish growers with cultivars adapted to their local conditions. The Spanish National Barley Breeding Program was set out by four public research organizations with this purpose. These four centres are placed at the most representative barley growing regions of Spain. The program is conducted in a joint manner by four public research bodies: Instituto Técnico Agronómico Provincial (ITAP) in Albacete, Instituto de Investigación y Tecnología Agroalimentarias (IRTA) in Lleida, Instituto Tecnológico Agrario de Castilla y León (ITACyL)



**Figure 1.** Location of the testing sites of the Spanish National Barley Breeding Program. Provinces (in grey) and locations (in black) hosting field trials.

in Valladolid and Estación Experimental de Aula Dei (EEAD-CSIC) in Zaragoza (Fig. 1).

The main objectives of this study were to study the progress and the selection efficiency in the Spanish National Barley Breeding Program, and to verify if this progress occurred uniformly across the four provinces of the program. Also, we wanted to have a general assessment of the extent and impact of genotype-by-environment interaction (GE) of grain yield in the final stages of the program. This study will focus on grain yield, the main target of the breeding program, but also on its relationship with flowering date. Flowering date is one of the most important traits for improving crop productivity and adaptation (Lawn *et al.*, 1995; Laurie, 2009; Brachi *et al.*, 2010), and is a primary objective of all breeding programs around the world.

## Material and methods

### Program description

The breeding program follows a strict pedigree scheme. Lines are extracted from the F<sub>2</sub>, and advanced up to the F<sub>10</sub> following a head-row system. Early gen-

Abbreviations used: G (genetic gain); GE (genotype-by-environment interaction); GL (genotype-by-location interaction); GLY (genotype-by-year-by-location interaction); GY (genotype-by-year interaction); H (the realized heritability); REML (restricted maximum likelihood); S (selection differential).

eration testing takes place from F3 up to F5, independently at each site. F6 is the first generation of joint testing where the lines from the four provinces are merged together for testing. The advanced trials start in F7 and continue up to F10. The number of lines selected is reduced at each generation.

At each province, several locations were used for testing (Fig. 1). In Albacete two trials were carried out in the same location: Albacete dry-land (A1) and Albacete irrigated (A2). In Lleida, four locations were used: Artesa (L1), Bell-lloc (L2), Gimennells (L3) and Solsona (L4). In Valladolid, several locations were used: Castronuevo (V1), Geria (V1), Villabañez (V1), Zamadueñas (V1), Villahoz (V2), Ceinos (V3), La Espina (V4) and Macotera (V5). At Valladolid, four locations near the capital city were used in different years. These locations were close enough to each other to be considered as a single location, V1. And in Zaragoza two locations were used: Sádaba (Z1) and Vedado (Z2). For two years, a location from a neighboring province, Navarra, was used. This was coded as Z3, since it was close to the locations from Zaragoza (Fig. 1). Not all locations were used every year. Trials were rotated between locations, with the exception of Albacete, and usually there were two trials grown per province and year.

All the locations under study are non-irrigated locations, except Gimennells (L3), where irrigation was provided as needed to avoid losing the trial when drought was severe, and Albacete irrigated (A2), which was always under irrigation.

The temperature in the locations under study show patterns typical of the Mediterranean climate, but with

some differences from location to location. Long term averages for temperature values were collected from the nearest meteorological stations to the locations under study.

## Data set

The data of this study were collected from the advanced stages of the Spanish National Barley Breeding Program. The analysis focuses on the advanced generations of the program, with a low number of lines per generation (Table 1). In these advanced trials, grain yield was the main selection criterion. The data set was gathered from 163 trials corresponding to generations F8, F9 and F10 carried out from 1998 until 2008. A total of 349 advanced lines were studied during that period. Out of these, 327 were recombinant inbred lines derived from 197 hybridizations, and 22 were double haploid lines. Besides, up to 24 check varieties were evaluated in the trials (Table 1).

The trials of the advanced generations followed an alpha-lattice of variable block size, with three replications, embedded in a randomized complete block design, with several test lines and checks. Each plot occupied 7.2 m<sup>2</sup> (6 m × 1.2 m), with either 6 or 8 rows. This area was modified for this study to 10.5 m<sup>2</sup> (7 m × 1.5 m) to take into account border effects.

The traits considered were: raw grain yield (in kg ha<sup>-1</sup>) at 10% moisture; relative grain yield for each line, expressed as the percentage of the average grain yield of the checks present at each particular trial; and flowering date, recorded as number of days from January

**Table 1.** Summary of lines and checks used in the advanced generations trials at the Spanish Barley Breeding Program

Years	Common checks	F8	F9	F10	F8	F9	F10
		Additional checks			Test lines		
1998	Barbarrosa, Alpha, Zaida	5	2	7	25	15	14
1999	Barbarrosa, Alpha, Zaida	2	2	6	20	11	7
2000	Barbarrosa, Alpha, Zaida, Graphic	1	6	6	23	4	4
2001	Barbarrosa, Alpha, Zaida, Graphic	2	6	6	30	6	1
2002	Barbarrosa, Alpha, Zaida, Graphic	1	1	2	23	15	6
2003	Barbarrosa, Alpha, Zaida, Graphic	0	1	2	32	15	12
2004	Barbarrosa, Alpha, Zaida, Graphic, Hispanic	0	0	0	31	15	11
2005	Barbarrosa, Graphic, Hispanic, Cierzo	0	0	0	32	16	14
2006	Barbarrosa, Graphic, Hispanic, Cierzo	0	0	0	32	16	11
2007	Barbarrosa, Graphic, Hispanic, Cierzo	0	0	0	32	16	11
2008	Barbarrosa, Graphic, Hispanic, Cierzo	0	0	0	32	16	10
Total					312	145	101

1<sup>st</sup> when at least 2 cm of the awns were visible in 50% of the tillers of each plot.

The use of relative grain yield allows homogenizing the results among years and locations, and among analyses, therefore avoiding possible problems of scale due to differences in productivity across years and locations.

The data set is highly unbalanced because it was collected over 11 years, and the maximum period that any line stayed in the program was for three years. The advanced lines stayed in the program 1, 2 or 3 years, depending on the generation in which they were discarded. There were a few exceptions because some lines were introduced directly either in F9 or F10. For these lines, previous generations are missing. Also, a few lines were retained for additional years after F10, to get additional data before a final decision was made. To cope with the unbalancedness of the data, a mixed model approach (REML) was used, implemented in the software package Genstat 12 (Payne *et al.*, 2009).

The relative grain yield was used to estimate the progress in the Spanish National Barley Breeding Program. To calculate the averages for each generation at each main location and province, two separate analyses were calculated using mixed models, considering locations or provinces as fixed factors, whereas years and the interactions with years were considered as random factors.

To calculate selection differential, genetic gain and realized heritability, the procedure of St. Martin & McBlain (1991) was used. The procedure is a test in which a set of lines evaluated in a generation is paired with a test in the next stage, in which selections from the set are re-evaluated. The procedure was adjusted to allow for the presence of different checks in the consecutive generations, which occurred in our data in some occasions. These calculations were done for the two selection steps available: F8-F9 and F9-F10, according to these expressions:

$$\begin{aligned} S &= (X_s - X) \cdot 100 \\ G &= (X'_s - X) \cdot 100 \\ H &= G/S \cdot 100 \end{aligned}$$

where  $S$  is selection differential,  $X_s$  is the mean of the experimental lines selected from the first stage (F8 or F9) for testing in the successive second stage (F9 or F10),  $X$  is the mean of all experimental lines evaluated in the first stage (F8 or F9),  $G$  is genetic gain,  $X'_s$  is the mean of the experimental lines selected from the

first stage and evaluated in second stage (F9 or F10) and  $H$  is the realized heritability.

To calculate the components of variance, the complete data set was used, but divided into two groups, according to the presence of a minimum of three common checks among the trials. The first group contained 242 genotypes and 12 locations during 7 years (1998-2004) and the second group contained 163 genotypes and 11 locations, during 4 years (2005-2008), with some genotypes represented in the two analyses. Even though the data were unbalanced, the presence of a minimum of common checks in all trials of each group of years, plus the presence of some breeding lines for two or three consecutive years, provided enough replication of genotypes to allow an estimation of variance components.

The components of variance were calculated using the original raw grain yield data. Genotypic averages per locations were used for these analyses, as these are the data available for all trials. For the sake of this analysis, genotypes, locations and years were considered as random factors, as they can be regarded as random samples of all possible levels of each factor that can be encountered for barley growing in Spain.

To break-down the GE into 'Genotype  $\times$  Province' and 'Genotype within Provinces' interaction, two homogeneous series of genotypes repeated for two years were identified, *i.e.* 1998-1999, 2001-2002, 2003-2004, 2005-2006, and 2007-2008. Each series contained a group of genotypes tested in the same environments (combinations of years and locations) at two consecutive years. Analyses of variance (ANOVA) for relative grain yield were calculated for two series of balanced groups of genotypes. The first series contains the groups of lines in generations F8 and F9 at two consecutive years. And the second series contains groups of lines in generations F9 and F10 at two consecutive years. Each series contains five groups.

Linear regression was used to calculate the regression coefficient between flowering date and relative grain yield using the appropriate routine in Genstat 12.

## Results

In all the advanced trials (F8, F9 and F10), several outstanding cultivars were included as checks. The number of checks varied from year to year, and also between locations, especially during the first years (Table 1). The checks were gradually changed along



the years, always aiming to include the best cultivars available, combining spring and winter cultivars. A set of common checks was maintained across locations, ranging from 3 to 5 checks per year. These common checks were chosen because they were used in the national trials for cultivar registration, and kept shifting as these cultivars were being renewed.

The selection pressure applied from generation to generation was not constant across years and, overall, was stronger at F8 (46% of lines promoted to F9) than at F9 (70% of lines promoted to F10).

The number of lines tested varied among years, with an average of 28, 13, and 9 lines tested in F8, F9, and F10, respectively (Table 1). In the period under study, a minimum of 31 genotypes were evaluated every year at advanced trials, at a minimum of three locations. Over the years, the program has become more stable in terms of number of checks and lines under test at every generation.

In the data set under study there was a large range in the grain yields recorded, from a minimum of 842 kg ha<sup>-1</sup> to a maximum of 6,974 kg ha<sup>-1</sup>. The overall mean for the entire period was 3,687 kg ha<sup>-1</sup>. The productivity levels were quite different between locations. The least productive location was Albacete dryland (A1). The highest yielding location was Bell-lloc (L2). Productivity was also high in Gimennells (L3), Albacete irrigated (A2) and Macotera (V5), intermediate in Ceinos (V3), V1 (Castronuevo, Geria, Villabañez and Zamadueñas), Sádaba (Z1), Vedado (Z2) and Artesa (L1) (Table 2).

Across years, average productivity was less variable, always in the medium productivity range, from a minimum of 3,200 (2005) to a maximum of 4,890 kg ha<sup>-1</sup> (2007). Productivity was higher in the last two years, in which it surpassed 4,000 kg ha<sup>-1</sup>.

To estimate the progress due to selection, we needed to combine the results of years and locations, even though they had different productivity levels. For this purpose we used the relative yield, because it does not fluctuate across years and locations. Rather, it presents values always around 100, and so the values for all trials can be easily combined, although sacrificing the overall productivity perspective.

The averages, for each generation, at each main location and province were calculated in two separate analyses (one for locations, one for province, Table 2). Some of the locations were used only occasionally (L4, V2, V4 and Z3). Their inclusion in the analyses increased largely the unbalancedness of the data, therefore affecting the quality of any estimates derived from

**Table 2.** Grain yield expressed as percentage of checks and average productivity in different locations and provinces, in the last three generations (F8, F9 and F10) of the Spanish Barley Breeding Program from 1998 to 2008. Averages across provinces and overall average, calculated with REML, in bold type

	F8	F9	F10	Grain yield (kg ha <sup>-1</sup> )
A1	96.3	101.4	96.5	2,683
A2	98.1	101.4	105.9	4,517
<b>Albacete</b>	<b>96.0</b>	<b>100.7</b>	<b>100.8</b>	<b>3,626</b>
L1	101.2	101.9	102.5	3,012
L2	102.3	107.3	107.4	4,966
L3	99.4	94.5	98.4	4,636
<b>Lleida</b>	<b>101.1</b>	<b>101.3</b>	<b>102.8</b>	<b>4,179</b>
V1	99.0	100.8	97.4	3,478
V3	94.9	106.4	102.9	3,844
V5	98.6	101.6	105.3	3,900
<b>Valladolid</b>	<b>99.0</b>	<b>102.2</b>	<b>102.8</b>	<b>3,685</b>
Z1	97.2	101.6	105.4	3,138
Z2	101.8	110.7	113.5	3,021
<b>Zaragoza</b>	<b>97.6</b>	<b>103.3</b>	<b>107.5</b>	<b>3,109</b>
<b>Total</b>	<b>98.9</b>	<b>102.8</b>	<b>103.5</b>	

them. These minor locations were removed from most analyses to reduce the overall unbalancedness, and get better estimates of the factors studied for the main testing locations (Table 2).

The comparison of the relative yields at the 10 main locations (during 11 years) indicated that there was progress at most locations over the three generations (Table 2). Overall, progress was evident. The means for the three advanced generations were different, F8 presenting the lowest mean and F10 the highest one (Table 2). At F8, the overall grain yield was already close to the level of the checks (98.9), and by F10 the outstanding lines clearly surpassed the checks by 3.5%.

Looking at the results of the provinces, in general, progress from F8 to F10 was observed at all four provinces, meaning that the program was successful overall. Differences among provinces were also apparent. The overall progress was larger at Zaragoza and Albacete, and smaller at Lleida and Valladolid.

Progress also differed at the single location level. In F8, only three of the ten main locations reached the yield level of the checks, whereas in F10 these figures were reversed. At F9, the progress was even more evident, as the lines surpassed the checks in all but one location. The highest progress was observed in Z2, where F10 lines surpassed the checks by 13.5%. The progress was large and consistent at the two Zaragoza

**Table 3.** Selection differential (S), genetic gain (G), and realized heritability (H, expressed as percentage of expected gain) calculated for groups of lines in two sets of consecutive generations (F8-F9 and F9-F10) tested in the same locations

	1 <sup>st</sup> generation		2 <sup>nd</sup> generation	S	G	H
	All lines	Selected lines				
F8-F9	95.9	102.1	102.0	6.24	6.09	97.6
F9-F10	99.9	106.1	100.2	6.28	0.37	5.9

locations, and smaller at the Lleida locations. In three locations, A1, V1, and L3 the average F10 lines did not reach 100, *i.e.*, their average did not surpass the checks’.

The selection differential (S), genetic gain (G), and realized heritability (H) were calculated for the two selection steps available: F8-F9 and F9-F10. The calculations of S, G and H, were done for sets of lines that were tested in the same location in consecutive years (Table 3). The figures indicate an excellent realized heritability was attained for the F8-F9 step, whereas it was low for the F9-F10 step.

The evaluation of a breeding program that includes testing in multi-environment trials must take into account which are the factors that cause genotypic variation. The relative size of these components will allow an assessment of the appropriateness of the testing strategies.

The components of variance were calculated for two subsets of data (Table 4), made of the sets of years that presented several common checks (Table 1). The component of variance for the error was calculated at each individual trial analysis, for each generation at each year and each location. These analyses are routinely done in the Spanish National Barley Breeding Program. The original data for all replicates was not always kept, but the original analyses of variance for most of them are still available. So, the error component of variance was calculated as an average of the error term corresponding to individual trials, weighted according to the degrees of freedom of each individual analysis.

After calculating the components of variance for the two groups independently, a weighted average was calculated for the components of these groups, relative to the number of units which were used in each analysis. This weighted average was assumed to represent the best estimate of the components of variance for the entire dataset under study.

The environmental components of variance were large. ‘Location’ was rather large, and ‘Year’ was highly variable. But, overall, ‘Year × Location’ was the

dominant environmental component, which meant that the productivity of locations varied largely between years (Table 4).

The calculations of broad-sense heritability in the two analyses were 0.70 and 0.75 respectively, with a general average of 0.71 over the two analyses. These values suggest the possibility to perform selection effectively, though the response may be low some years due to a relatively low genotypic variance (Table 4).

An important variance due to ‘Genotype’ was present in the two analyses. The variance of the GL was larger than that of the GY in the two analyses. This suggests that GE shows some geographic trend. But the three way interaction (GLY) was larger or even much larger in each analysis, meaning that the geographic trends vary from year to year and are, therefore, unpredictable.

The GE was broken down into ‘Genotype × Province’ and ‘Genotype within Provinces’ interaction for the two balanced series of genotypes and environments. The analyses of variance for these groups are shown in Table 5. In most of the groups the variance of ‘Genotype × Province’ and the ‘Genotype within Provinces’

**Table 4.** Components of variance for grain yield in the Spanish Barley Breeding Program. The two periods were chosen according to the presence of sets of common checks

Random term	1998-2004	2005- 2008	Weighted average
n (units)	2,172	1,865	
Year (Y)	0	1,657,120	765,551
Location (L)	1,073,410	1,158,223	1,112,592
Y × L	2,333,147	1,960,767	2,161,116
Genotype (G)	69,426	58,736	64,487
G × Y	95,698	26,570	63,762
G × L	145,824	34,329	94,316
G × L × Y	295,777	361,766	326,262
Error	208,858	235,394	224,711
Broad-sense h <sup>2</sup>	0.70	0.75	0.71

**Table 5.** Summary of the genotype-by-environment interaction factor for ten different analyses of variance for relative yield. The analyses were performed for ten sets of genotypes, which were balanced over two-year trials, either F8 and F9 or F9 and F10

Years	Generations	Mean squares	
		Genotype $\times$ Province	Genotype within Province
1998-1999	F8-F9	253 <sup>ns</sup>	160
1998-1999	F9-F10	126 <sup>ns</sup>	234
2001-2002	F8-F9	91 <sup>ns</sup>	119
2001-2002	F9-F10	224 <sup>ns</sup>	141
2003-2004	F8-F9	182 <sup>ns</sup>	149
2003-2004	F9-F10	201 <sup>ns</sup>	190
2005-2006	F8-F9	95 <sup>ns</sup>	86
2005-2006	F9-F10	87 <sup>ns</sup>	111
2007-2008	F8-F9	102 <sup>ns</sup>	85
2007-2008	F9-F10	125*	69

terms were rather similar, and in 9 out of 10 of the groups the variance of ‘Genotype  $\times$  Province’ (tested against the residual GE, *i.e.*, the ‘Genotype within Provinces’ term) was not significant. This means that, actually, the provinces did not explain much of the GE.

Flowering time data were recorded at most of the locations and years. When flowering date was recorded for a given location, it was done for all trials in that location. The averages of flowering dates for the three

generations at all locations were calculated with a mixed model using REML, considering ‘generation’ and ‘location’ as fixed factors, and ‘year’ and its interactions as random factors (Table 6).

Lleida presented the earliest flowering dates, whereas the latest one was Valladolid. Zaragoza and Valladolid showed the widest flowering time ranges (Table 6). The flowering date means were almost constant across locations and provinces for the three generations F8, F9 and F10. The range of flowering dates became narrower with increasing generations, but this could be an effect of sample size.

The regression analysis between grain yield and flowering date was used to further analyze the possible presence of trends in the data. The regression coefficient was calculated using the relative yield and flowering time data of the genotypes under study (lines and checks). The regression coefficient was calculated for all trials run at each year-location combination (usually F8, F9 and F10, taking advantage of the fact that all three trials were commonly sown on the same date). The regression coefficients between relative grain yield and flowering time were low (Table 7). Even though it was statistically significant in some trials, due to the large number of points, the slope of the regression line was almost flat. In some trials (16, *i.e.* about one third), there was a significant negative relationship between relative grain yield and flowering time.

**Table 6.** Summary of number of lines, flowering date means, minimum, maximum, expressed as the number of days from January 1<sup>st</sup>, and range of flowering dates for the breeding lines under study (checks excluded), by location and province. Means are REML estimates, whereas minimum, maximum and ranges were calculated with raw values. Averages across provinces and overall average in bold type

	Lines	Mean	Minimum	Maximum	Range
A1	103	118.3	101	129	28
A2	101	121.7	105	140	35
Albacete	121	120.5	101	140	39
L1	119	114.1	96	127	31
L2	77	104.8	93	120	27
L3	99	106.3	89	119	30
Lleida	177	106.8	89	127	38
V1	93	126.2	110	142	32
V3	23	126.7	120	135	15
V5	121	120.0	108	135	27
Valladolid	135	123.3	108	142	34
Z1	159	120.4	108	141	33
Z2	69	114.1	96	130	34
Zaragoza	159	115.9	96	141	45
Total		117.3	102.6	131.8	29.2

**Table 7.** Results of the regression analyses between relative yield and flowering time in the trials during the period of the study

Location	Year	Generation	b	R <sup>2</sup>	Constant	F pr.
A1	2003	F8-F10	-0.81	0.039	191	0.093
A1	2004	F8-F10	-0.30	0.009	135	0.427
A1	2005	F8-F10	-2.32	0.187	376	<0.001 **
A1	2006	F8-F10	-1.30	0.129	248	0.002 **
A1	2007	F8-F10	-2.44	0.106	412	0.006 **
A2	2003	F8-F10	-1.87	0.059	351	0.038 *
A2	2004	F9-F10	0.68	0.018	27	0.440
A2	2005	F8-F10	-0.12	0.002	112	0.748
A2	2006	F8-F10	-0.26	0.005	128	0.564
A2	2007	F8-F10	-3.32	0.127	523	0.002 **
A2	2008	F8-F10	0.34	0.006	56	0.536
L1	2003	F8-F10	-2.20	0.119	358	0.003 **
L1	2007	F8-F10	1.16	0.163	-25	< 0.001 **
L1	2008	F8-F10	0.89	0.075	-7	0.022
L2	1999	F8-F10	-1.63	0.187	270	< 0.001 ***
L2	2002	F8-F10	0.15	0.003	86	0.694
L2	2004	F8-F10	-1.46	0.052	272	0.053
L2	2006	F8-F10	-2.09	0.287	306	< 0.001 **
L3	1998	F8-F10	-1.00	0.030	209	0.135
L3	2000	F8-F10	0.33	0.010	57	0.517
L3	2001	F8-F10	0.16	0.002	87	0.746
L3	2005	F8-F10	0.13	0.001	80	0.781
L3	2007	F8-F10	0.08	0.002	89	0.709
V1	1998	F8-F9	-0.32	0.016	143	0.373
V1	2002	F8-F10	-0.10	0.001	111	0.788
V1	2005	F9-F10	-2.82	0.417	440	< 0.001 **
V1	2006	F8-F10	-0.69	0.016	195	0.312
V1	2007	F9-F10	-1.24	0.134	258	0.043 *
V1	2008	F8-F10	-0.26	0.013	129	0.345
V3	1999	F8-F10	-0.80	0.065	199	0.056
V4	1998	F8-F9	-0.77	0.060	219	0.079
V5	1999	F8-F10	-0.23	0.039	125	0.142
V5	2000	F8-F10	-1.56	0.365	296	< 0.001 **
V5	2002	F8-F10	0.14	0.004	82	0.619
V5	2005	F8-F10	-2.47	0.146	397	0.003 **
V5	2006	F8-F10	-3.30	0.321	485	< 0.001 **
V5	2007	F8-F10	-0.18	0.006	118	0.532
V5	2008	F9-F10	1.82	0.187	-110	< 0.001 **
Z1	2002	F8-F10	0.56	0.054	35	0.074
Z1	2003	F8-F10	-2.48	0.200	395	< 0.001 **
Z1	2004	F8-F10	-1.30	0.222	274	< 0.001 **
Z1	2005	F8-F10	-0.02	0.000	98	0.919
Z1	2006	F8-F10	-0.55	0.059	159	0.041 *
Z1	2007	F8-F10	-0.85	0.042	202	0.087
Z2	2001	F8-F10	-0.12	0.002	116	0.775
Z2	2003	F8-F10	0.25	0.001	82	0.750
Z2	2004	F8-F10	-0.45	0.006	174	0.539
Z2	2007	F8-F10	-0.46	0.037	155	0.107

\*, \*\*, significant at  $p \leq 0.05$  and  $p \leq 0.01$  respectively.



## Discussion

The progress associated with selection, the relationship between flowering date and grain yield, and the existence of GE have not been studied previously in the Spanish National Barley Breeding Program. The success of the program is evident, based on its capacity to produce improved cultivars, which are being readily adopted by the industry and the producers. Nevertheless, a systematic retrospective analysis may offer clues about the effectiveness of the practices used, and help to identify possible weaknesses of the program.

It is assumed that each set of checks marked, at each year and location, the threshold of agronomic excellence for the program. Therefore, the overall relative yield means (Table 2) indicate a significant progress in the barley breeding program over the period studied. The difference between all three generations was remarkable, and in the end surpassed the yield of the checks. It seems that the overall progress slowed down after F9, however, as there was an increase of only 0.7% from F9 to F10 compared to 3.9% from F8 to F9. This may have been affected by the lower selection pressure applied from F9 to F10 (Table 1).

Another conclusion from the overall means is that the program already achieved a good productivity level at F8, with a mean performance quite close to the checks (98.9%). A similar trend in the performance of selected lines and check cultivars has been reported by Khalil *et al.* (2004) in a wheat breeding program. This may be the result of an efficient selection over the generations up to F8 or, alternatively, could mean that the productivity level achieved for the materials in the program is high from the very beginning. It is not inferred from the data which of these hypotheses is more likely. But the fact that most of the parents currently used in the program are recycled advanced lines suggests that the program may be reaching a mature stage, in which productivity level is optimized across all generations.

The true gain attained in the program is probably higher than the calculated for the relative yields. As the checks were gradually replaced over the years, it can be safely assumed that the yield level of the checks also rose over the years, as the new checks replaced older cultivars that became obsolete. In consequence, the gain calculated for relative yield is most likely an underestimation of the true gain in kilograms per hectare.

At the province level, there was higher progress in Albacete and Zaragoza, compared to Lleida and Val-

ladolid. The small progress in Lleida and Valladolid may have been partially caused because, at these provinces, the F8 already showed a very high grain yield level, and subsequent progress could have been more difficult to attain. Though the gain in Albacete was apparent, the final yield level at F10 barely reached the level of the checks, whereas at the other three provinces, F10 lines level clearly exceeded the checks.

Gain from selection was apparent at most locations. In three locations, F10 relative yield was below 100, *i.e.*, the program was less effective in finding superior cultivars for these locations. The case of V1 was not surprising, as it was actually a conglomerate of different locations close to Valladolid city and, in consequence, a larger effect of GE (lowering genetic gains) is expected. On the other hand, the case of A1 (Albacete dry-land) is worrying, as it seems that the program is not achieving its objective at the lowest yielding location. The low progress at this location affected the result of Albacete as a whole, and explains the unsatisfactory overall results at this province. It can be speculated that the program is not addressing properly the adaptation to the poorest growing conditions. To test this, we calculated a correlation coefficient between the program progress (the difference between F8 and F10) and the mean grain yield at the 10 main locations. The  $r$  value was just -0.12, indicating that the relationship between response to selection and productivity level was probably negligible. Finally, there is no plausible explanation for the low progress at L3.

Positive genetic gains from F8 to F9 were found (as in the studies of Khalil *et al.*, 2004, 2010). But it was very low, almost negligible, from F9 to F10, though this was affected by other factor that will be discussed below. In any case, this indicates a lower effect of selection after F9. There were some lines tested for more than one year in F10. These lines used to be the best lines of the trial, that were maintained in the program for some additional years before taking the final decision of releasing them as cultivars or recycling them as parents. This was the reason of the apparently different results for the F10 in Table 2. In Table 3, the results of only the first year of F10 evaluation were presented. Actually, the lines that were kept in the program for additional years at the F10 had a relative yield above 105 in the second and third years of evaluation. Their absence in the calculations of realized heritability swayed the overall F10 average slightly downwards. The reasons for not reaching a realized heritability of 1 are the presence of error and of GE.

Regarding components of variance, 'Year' variance was very different between the two analyses done (Table 4). This is explained by the rather constant yearly averages observed during the first period analyzed (1998-2004), compared to the highly variable averages observed in the second period (2005-2008, Table 4). This was not unexpected, as large yearly fluctuations are common in Mediterranean environments (Turner, 2004). Genotypic variance was detected in the two analyses performed, meaning that there were true genotypic differences still at this stage of the program. It had comparable size to the GL and GY interactions. In a similar study focused on a wheat breeding program, Roozeboom *et al.* (2008) found a genotypic variance almost twice as large as the GL and GY variances. Similar figures were found by Thomason & Phillips (2006), for wheat breeding in Virginia. Their studies are relevant to ours because they were also testing advanced materials (candidate cultivars) in large geographical areas with highly variable environments (especially Roozeboom *et al.*, 2008). This shows that the situation for the Spanish barley breeding program presents even higher challenges, as the interactions involving the 'Genotype' factor were higher.

GL in the data was rather high, indicating the presence of a geographical factor in the GE. When this happens, the breeders are confronted with the issue of whether the program should target wide adaptation, or it should be split between different locations due to the high GL interaction. But the results in the two analyses comprising the entire 11 years (Table 4) indicate that the 3-way interaction, between genotypes, locations and years was the principal source of variance. Therefore, the geographical patterns varied between years and were not predictable. Hence, a split of the program based on more stable geographic sub-zones is not advisable.

Consistent with this, it is observed that there was almost no Genotype  $\times$  Province interaction (Table 5). Therefore, whatever factors were causing GE in this dataset, they seemed not related with geographical division at the province level. This finding reassures that the current strategy, combining the results of the four provinces is appropriate. Cullis *et al.* (2000) found a similar situation when analyzing series of variety tests conducted for several crops in Australia. They found that classical geographic zonation had little meaning under the light of actual variance components calculated for them.

The presence of locations from all provinces ensures a good coverage of all GE situations possible. In other

words, the representativeness of the locations is good. It may be argued that the two Albacete locations (actually, two trials in the same location) are redundant to some extent. But the very distinct results observed in response to selection between A1 and A2 (Table 2) suggests that these two trials are probably giving different, non-overlapping information.

The changes in flowering date means and ranges indicate that, even though this trait has undergone several rounds of selection by this stage of the breeding program, there was still a slight selection towards earliness from F8 to F10 (Table 6). There was a spread of flowering dates across locations, proportional to the mean temperatures over the growing season, with colder locations (from Valladolid) reaching flowering later than warmer locations (for instance, L2 and L3). A dynamic relationship of flowering date with barley yield in Spanish environments was already found by Cuesta-Marcos *et al.* (2009). Though some water stress is almost always present in our conditions, timing and intensity of this stress varies widely. Therefore, it is not surprising that the relationship between flowering date and yield changed depending on the environment. The regression coefficients between relative grain yield and flowering time were, in general, rather low (Table 7) indicating that the relationship between yield and flowering time overall was weak in the locations under study at this advanced stage of the program. This relationship would possibly be more tight if the selection up to F8 had not removed already the most early and, especially, late genotypes.

In summary, there was progress due to selection over the last generations of the Spanish National Barley Breeding Program. Grain yield increased from F8 to F10, surpassing the level of the checks. We can conclude that the program is reaching its main goal of producing and identifying superior barley genotypes with high yield potential and stability suitable across all Spanish barley growing regions. The effectiveness of selection was satisfactory across all four provinces, though differences were observed among particular locations. It was also more effective up to F9, whereas there was little gain in the last generation.

These results also suggest that it would be unpractical to run separate breeding programs for separate provinces or locations (either considering an entire program or just the last generations). If we had found clear differences in GE among provinces, the situation might have been different, as provinces are large geographical units, which may justify additional efforts. But the struc-

ture of the components of variance and the absence of a stable geographic structure of the GE, it seems sensible that the program continues with the same geographic structure, using the same provinces and locations.

The definitive proof of the success of a breeding program is the adoption of the varieties released by the industry. Cultivars Cierzo, Estrella and Yuriko, released over the last five years performed very well in independent trials, and are currently under exploitation by three different companies.

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