PATTERNS OF GENETIC DIVERSITY ASSOCIATED WITH PHENOLOGICAL TRAITS IN THE SPANISH BARLEY CORE COLLECTION

Casas AM*[1], Ciudad FJ[2], Moralejo MA[3], Montoya JL[2], Yahiaoui S[1], Lasa JM[1], Gracia MP[1], Igartua E[1]

[1] Department of Genetics and Plant Production, Estación Experimental de Aula Dei, CSIC, PO Box ,50080 -Zaragoza, Spain

[2] Servicio de Investigación y Tecnología Agraria, Zamadueñas, Crta. Burgos km 118, PO Box 172, 47080-Valladolid, Spain

[3] Escuela Técnica Superior de Ingenieros Agrónomos, Universitat de Lleida, Avda. Alcalde Rovira Roure 177, 25198-Lleida, Spain

* acasas@eead.csic.es

EUCARPIA
Cereal Section Meeting
Salsomaggiore 2002

INTRODUCTION. MATERIALS AND METHODS

This is a study on genetic diversity patterns in barley, and their relationship with phenological development, an important component of cereal adaptation. A collection of old Spanish landraces (the Spanish Barley Core Collection), 153 6-row, 11 2-row, and a set of 57 2- and 6-row cultivars from other origins, mostly from other European countries (Germany, France, Netherlands, Belgium, Great Britain, Denmark, Sweden, Norway, Finland, Italy, Greece, Yugoslavia) were analysed for the presence of SSR (Pillen et al., 2000; Ramsay et al., 2000; Macaulay et al., 2001) and one STS. Spanish accessions are inbred lines derived from a single head from each original population. SSR band sizes were determined according to a 30-330 pb ladder (AFLP ladder, Invitrogen), analysing the gels with the image analysis software Diversity Database (BioRad). *NTSYS* (Rohlf, 2000) software package was used to graphically depict the relationships between accessions. The package *Structure* (Pritchard et al., 2000) was used to assess the genetic profiles of the accessions, and to estimate the putative number of populations represented in these materials by a Bayesian approach. The responses of the accessions to temperature and photoperiod were evaluated in four greenhouse treatments, combining presence or absence of vernalizing temperatures with long or short photoperiods. Association analyses of markers with phenotypic traits were carried out by multiple regression with SAS (SAS Institute, 1985), taking into account the population structure found in the data.

RESULTS AND DISCUSSION

Classification

The relationships between the accessions were represented with a dendrogram (Fig. 1). There was a clear separation between 6-row (top four groups) and 2-row types (bottom large group), with a small group of CIMMYT-ICARDA materials clustering separately. Most Spanish accessions fell into two big groups not including foreign accessions. Other European 6-row types fell into two groups, together with some Spanish accessions, apparently classified according to type of cultivation (winter or spring). The Spanish subgroups presented differences in allele frequencies at a remarkable number of loci, among which the most notable was *MWG699*, an STS proposed as a marker of domestication history (Tanno et al.,1999, 2002).

The Bayesian approach implemented in the *Structure* package suggested the presence of, at least, four distinct populations, briefly described in Fig. 2. A majority of accessions were clearly classified as belonging to a specific group (above 0.75 probability of belonging to a single group), whereas a minority (about 25%), showed mixed origins.

The distribution of accessions in these newly formed four groups, compared with the ascription to *a priori* germplasm groups (Table 1) reveals overall good agreement among the two classifications. The most striking feature is the division of the Spanish 6-row accessions into two groups, same as in Fig. 1. Spanish groups again showed assimmetric distribution of *MWG699* alleles.

The genetic diversity patterns of Spanish barleys are consistent with the existence of two different genetic sources or founding stocks, identified by the *MWG699* A and D patterns (colour-coded in Fig. 1), partially admixed through the years, but still identifiable. Another possible explanation would be the presence of multilocus associations induced by adaptation to different environmental conditions. This explanation is supported by the seemingly non-random association of collection sites latitude and altitude with genetic cluster membership (Fig. 3). These two hypotheses are not mutually exclusive.

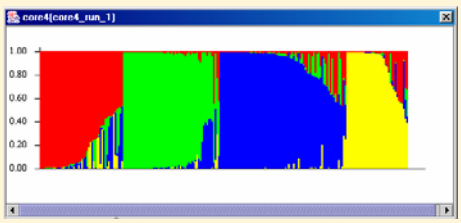

Figure 2. Summary plot of Q estimates (proportion of each individual's genome originating from each inferred population with *Structure*).

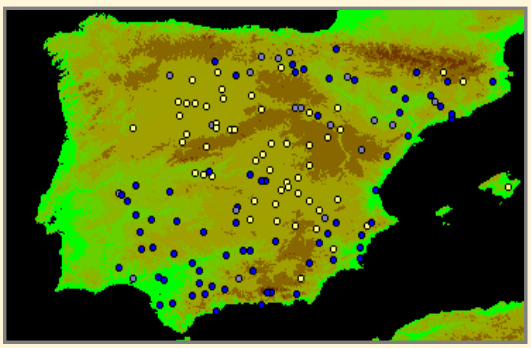

Figure 3. DIVA-GIS (Hijmans et al., 2002) plot of collection sites for the 6-row Spanish accessions. Background colors represent altitude. Population 1 represented with gray symbols; population 2,blue; population 3, yellow.

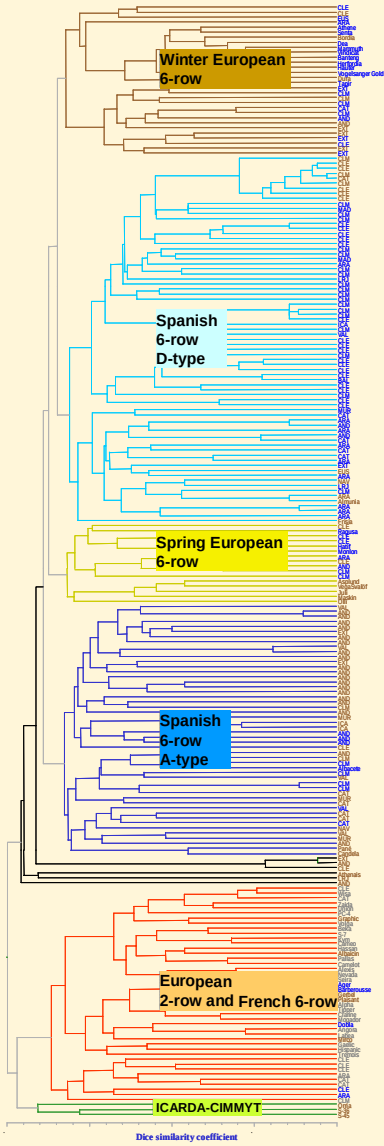

Figure 1. UPGMA dendrogram for barley accessions, using the Dice distance, on a data matrix with 50 SSR and 1 STS. Groups are colour-coded. The most relevant characteristics for each group, or the main identifiable germplasm group are also displayed. Genotypic labels are colour-coded for MWG type (A-brown, D-blue, K-gray).

Association analysis

The phenological development of barley plants under four temperature-photoperiod regimes was described as the number of leafs until the plants reached a particular stage. The no. of leafs until flag leaf appearance under the four treatments is presented in Table 2, as well as the sensitivities to temperature and photoperiod. There were significant differences among *Structure* inferred populations for all treatments except for *vernalization – long photoperiod*, the most inducive one. The two private Spanish groups (populations 2 and 3) presented some vernalization requirement. Population 3 presented some photoperiod sensitivity under long photoperiod, whereas pop. 2 presented values close to pop. 4, which includes a majority of spring types. The patterns observed suggest the presence of different reaction types between the four populations, which may have an adaptive meaning.

A multiple regression analysis of phenotypic traits on groups of each marker bands presented highly significant results for a majority of the marker-trait combinations (Fig. 4). Most of these associations disappeared when the population membership probabilities were introduced in the regression model. Some of the remaining associations make sense according to prior knowledge. The results offer great potential to identify regions responsible for Spanish landraces specific adaptations.

Table 2. Averages of phenological traits for four populations of barley accessions deduced from molecular diversity patterns with the package *Structure*.

	Total number of leaves produced at four different growing conditions				Vernalization and photoperiod effects calculated			
Population	Vernalization long photoperiod	Vernalization, short photoperiod	No vernalization, long photoperiod	No vernalization, short photoperiod	*VLP*	*VSP*	*PV*	*PNV*
1	6.8	13.5	11.0	15.0	4.2	1.5	6.7	4.1
2	6.8	11.6	12.9	12.6	6.0	0.9	4.8	-0.3
3	6.8	12.6	11.5	13.2	4.7	0.7	5.8	1.7
4	6.8	11.0	8.5	11.4	1.7	0.5	4.2	2.8
signification	ns	**	**	**	**	**	**	**
R^2	0.00	0.27	0.31	0.40	0.36	0.08	0.27	0.33

VLP: vernalization effect under long photoperiod
VSP: vernalization effect under short photoperiod
PV: photoperiod sensitivity in vernalized plants
PNV: photoperiod sensitivity in non-vernalized plants

Ns non-significant
** P<0.01

Table 1. Cross classification of germplasm group membership and populations deduced from the *Structure* clustering procedure. Relative frequencies.

	Number of	Populations inferred with STRUCTURE			
Germplasm groups	accessions	1	2	3	4
Spanish 6-row	153	0,142	0,372	0,474	0,012
Non Spanish 6-row	31	0,808	0,028	0,060	0,104
Spanish 2-row	11	0,177	0,060	0,086	0,678
Non Spanish 2-row	26	0,070	0,013	0,005	0,912

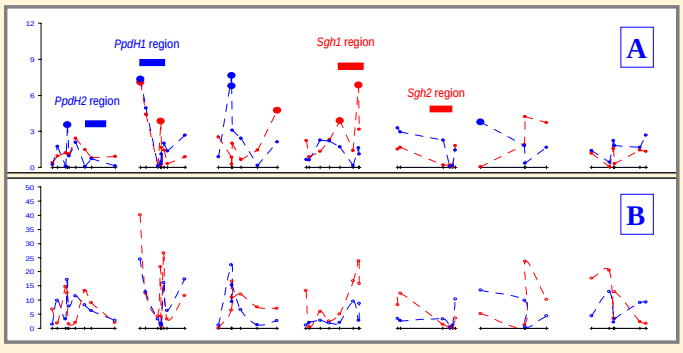

Figure 4. Genome scans for vernalization effect under long photoperiod (red lines) and no. of leafs under non vernalization – short photoperiod conditions (blue lines), for models including marker information only (model A), and population membership probabilities prior to introduction of marker information (model B). The magnitude on the Y–axis is the coefficient of determination for the markers.

REFERENCES

Hijmans R.J. et al. 2002. DIVA-GIS, v 2. International Potato Center, Lima, Perú.
Macaulay M. et al. 2001. Theor Appl Genet 102: 801-809.
Nei M. and Li, W.H. 1979. Proc. Natl. Acad. Sci. USA 76: 5269-5273.
Pillen K., et al. 2000. Theor Appl Genet 101: 652-660.
Pritchard et al. 2000. Genetics 155:945-959.
Ramsay L. et al. 2000. Genetics 156: 1997-2005.
Rohlf F.J. 2000. NTSYSpc ver. 2.1. Numerical taxonomy and multivariate analysis system, Exeter Software, New York.
SAS Institute. SAS Procedures Guide for Personal Computers, v 6 ed. Cary, NC.
Tanno K.I. et al. 1999. Hereditas 130: 77-82.
Tanno K., et al. 2002. Theor Appl Genet 104: 54-60.

ACKNOWLEDGEMENTS

This work was funded by the Spanish National Institute for Agronomic Research (INIA), project RTA01-088-C3-3. The fifth author was financially supported by a scholarship from the Agronomic Mediterranean Institute from Zaragoza (CIHEAM).

CSIC Junta de Castilla y León IRTA Universitat de Lleida