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The agronomic variability of a collection of sainfoin accessions

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Abstract

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial forage legume appreciated for its feed value and rusticity. Two types are characterized via their growth habit and persistence: the common form which does not flower in the sowing year, and the giant form that does. In order to evaluate the degree of belonging to either of these types, thirty-eight Spanish sainfoin accessions and six foreign cultivars (44 accessions) were studied. The study involved three trials of 36 plants per accession in three locations in the northeast of the Iberian Peninsula during 2002-2004. Two locations received no irrigation while one was irrigated. The variables analysed were: percentage flowering in the sowing year, autumn regrowth, stem length at the end of winter, and the capacity for summer regrowth after the first cut. A cluster dendrogram showed two large groups encompassing common or giant sainfoins. These could be divided into three subgroups each according to the degree of contamination or crossing between the two major groups. Two easily identified characteristics —percentage flowering in the sowing season, and the speed of regrowth after the spring cut— help in the classification of these plants into one or the other form, and could facilitate cultivar selection.

Additional key words: autumn regrowth, flowering, *Onobrychis viciifolia* Scop., Spanish ecotypes, summer regrowth.

Resumen

Variabilidad agronómica de una colección de procedencias de esparceta

La esparceta (*Onobrychis viciifolia* Scop.) es una leguminosa forrajera plurianual, apreciada por su valor nutritivo y rusticidad. Existen dos tipos caracterizados por su hábito de crecimiento y persistencia: 'común', que no florece el año de siembra, y 'gigante', que sí florece. Con el fin de determinar su grado de pertenencia a uno de los dos tipos, se estudió la variabilidad de 38 procedencias nacionales y seis extranjeras de la esparceta. El estudio se realizó en plantas individuales, utilizando 36 plantas por procedencia en dos localizaciones de secano y una de regadío en el noreste de la Península Ibérica, durante 2002-2004. Los parámetros analizados fueron: porcentaje de plantas florecidas a lo largo del año de siembra, desarrollo otoñal y capacidad de rebrote después del primer corte. El análisis cluster distribuyó las 44 entradas en dos grandes grupos, definidos como 'común' y 'gigante'. Estos grupos pudieron dividirse a su vez en tres subgrupos, según el grado de contaminación o de cruzamientos habidos entre dichos grupos. Dos de los caracteres evaluados, el porcentaje de plantas florecidas el año de siembra y la rapidez del rebrote tras el corte de primavera, pueden contribuir a clasificar las plantas como de uno u otro tipo y facilitar la selección de cultivares.

Palabras clave adicionales: crecimiento otoñal, ecotipos españoles, floración, *Onobrychis viciifolia* Scop., rebrote estival.

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial forage legume much appreciated by farmers and breeders for its rusticity, its ability to restore soil fertility, its feed value, and its non-bloating qualities (Delgado *et al*., 2002). In the Iberian Peninsula this species prefers sub-humid, cold semi-arid conditions at altitudes of > 600 m, and calcareous, little-fertile soils (Buendía Lázaro and García Salmerón, 1965).

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Abbreviations used: LSD (least significance differences), NS (non significant), SD (standard deviation).

Sainfoin is an allotetraploid species obtained from wild botanical forms (Badoux, 1965). Its cultivation was started at the end of the XVI century in the provinces bordering the Rhine Valley. Soon, the common and giant types (also called one- or two-cut types respectively) were selected: the first is characterized by its rusticity and persistence, the second by its erect growth habit, larger leaf size and vigour (which allows two cuts per year) (Gasparin, 1846). Common sainfoin does not flower in the sowing year while giant sainfoin does (Rees, 1932, cited by Michelena, 1983).

The sainfoin originally sown in Spain was the common type. Giant sainfoin was introduced experimentally by the Botanical Gardens of Madrid in 1791 (Muller, 1893). Since then there have been successive introductions, the most important being that promoted by the Ministry of Agriculture at the end of the 1960s. Thus, foreign giant sainfoins have mixed with native forms (Pujol, 1974). Local ecotypes may have been altered due to seed transfer occasioned by farmers and commercial enterprises. Part of the seed demand is met by seed imported from Eastern Europe countries, and this is contributing further to the contamination of local ecotypes. No breeding has been undertaken.

Given its feed, health and environmental properties, Sainfoin could play an important role in the restructuring of rainfed, low-yield cereal land into pastures for extensive production or other uses in the northeast of the Iberian Peninsula. It is therefore important to survey and collect local ecotypes and to undertake breeding work to produce improved cultivars.

This work studies the variability of a collection of 44 accessions.

Material and Methods

Thirty-eight Spanish accessions were obtained from seed production growers in 2001-2002 (Fig. 1; Delgado *et al.*, 2002). Six foreign cultivars were provided by research institutes and commercial enterprises. Accessions 1-9 were collected in Huesca province, 10-15 in Teruel, 16 and 17 in Zaragoza, 18-21 in Lerida, 22-25 in Palencia, 26 in Guadalajara, 27 and 28 in Gerona, 29-31 in Burgos, 32 and 33 in Logroño, 34-36 in Soria, and 37-38 in Castellón. The foreign accessions were number 39, i.e., cv. Yubilevna (provided by the Forage Research Institute, Pleven, Bulgaria); number 40, i.e., cv. Visnovsky (provided by the Research Institute for Fodder Crops, Troubsko, Czech Republic); number 41, i.e., cv. Fakir (provided by the Institut National de la Recherche Agronomique, Montpellier, France); number 42 cv. Korunga (provided by the Agricultural Faculty, Izmir, Turkey); number 43 cv. Incoronata (provided by the Instituto Sperimentale Agronomico, Bari, Italia); and number 44, an unknown cultivar from The Ukraine provided by Rocalba S.A. (Spain).

Figure 1. The Spanish provinces where sainfoin seeds were collected.

The study was conducted over 2002-2004 at three locations in the northeast of the Iberian Peninsula: at Latre (Huesca province, longitude 42° 25'N; latitude 0° 28'W), where no irrigation was provided, at Lagueruela (Teruel province, longitude 41° 3'N; latitude 1° 11'W), with no irrigation either, and at Zaragoza (longitude 41° 45'N; latitude 0° 47'W), where irrigation was provided. The climate and soil characteristics of these locations are shown in Table 1.

Sowing was performed in spring 2002. Thirty six plants of each of the 44 accessions were sown by direct drilling using a planting frame of 0.3×1 m for the rainfed and 0.5×1 m for the irrigated land; the plants were distributed as three replicates of 12 plants (a random block design was used). In the irrigated trials, water was applied by monthly flooding during the summer months. No fertilizers, insecticides or herbicides were applied; all weeding was performed by hand.

The following variables were recorded: percentage flowering in the sowing year, autumn regrowth [on a visual evaluation scale from 1 to 9 (least to most regrowth)], and average stem length (cm) at the end of the winter. These variables were recorded in the autumn-winter from 2003 to 2004. The capacity for summer

Table 1. Location of the three trials, and local climate and soil characteristics

	Lagueruela	Latre	Zaragoza
Longitude	41° 3' N	42° 25' N	41° 45' N
Latitude	1° 11' W	0° 28' W	0° 47' W
Altitude (m)	1,066	698	225
Maximum mean temperature (°C)	16.1	18.6	21.0
Minimum mean temperature (°C)	7.9	4.9	8.6
Absolute minimum temperature (°C)	–7	–11.0	–8.3
Annual rainfall (mm)	552.7	649.9	381.0
Texture	Clay-loam	Loam	Silty-loam
Water pH 1:2.5	8.11	8.15	8.26
Salinity CE 1:5 dS m^{-1}	0.24	0.21	0.41
Organic matter %	1.69	1.75	1.39
Phosphorus Olsen (mg kg^{-1})	8.38	7.03	4.16
Potassium (ammonium acetate) (mg kg^{-1})	148	120	190

regrowth 20 days after the first cut was also measured visually in 2004 (using the same 1 to 9 scale). These important, easy-to-measure variables have been recorded in different studies on the characterization of local sainfoin ecotypes (Michelena and Hycka, 1988; Prosperi *et al.*, 1994) and alfalfa (*Medicago sativa* L.) (Hidalgo, 1966; Delgado, 1986).

The flowering percentages were arcsine-transformed prior to statistical analysis. The normality of the distributions of the autumn and summer regrowth data were tested using the Shapiro-Wilk's test. All data were analysed by ANOVA. Accessions, locations and their interaction were regarded as fixed effects. Comparisons between means were performed using the LSD test. Correlations between variables were assessed by calculating Spearman correlation coefficients. Cluster analysis was performed using Ward's minimum variance clustering method (Sneath and Sokal, 1973). All statistical procedures were undertaken using the SAS statistical package (SAS, 2004).

Results

Table 2 shows the mean values of the variables for all 44 accessions at each location. Most of the characteristics analysed showed highly significant differences between accessions and locations (Table 3). The interaction *accession × location* had a highly significant effect on most of the studied variables.

The average percentage flowering in the sowing year was 48.1 ± 15.86% at Lagueruela, 44.5 ± 25.70% at Latre, and 42.7 ± 28.40% at Zaragoza; the range of percentage flowering was 0-100. Significant differences ($P < 0.05$) were seen between Lagueruela and Zaragoza, with the smallest flowering percentage obtained in the irrigated fields of Zaragoza. At the latter trial site only those plants flowering within the first productive cycle were counted because a cut was made during the full bloom stage due to the strong development shown by the plants. In the remaining trails under rainfed conditions, flowering plants were counted throughout the productive period because of the poor growth shown in the sowing year.

Significant ($P < 0.001$) differences between accessions were seen in terms of autumn regrowth (6.6 ± 0.43 points on the 1-9 scale at Lagueruela, 6.6 ± 0.3 at Latre, and 5.8 ± 0.56 in Zaragoza). The differences in autumn regrowth between the dry land (Lageruela and Latre) and irrigated land sites (Zaragoza) were also significant ($P < 0.05$), perhaps due to the different cutting systems used in each.

Significant differences ($P < 0.001$) were also seen between accessions in terms of the growth at the end of winter (13.2 ± 0.91 cm at Lagueruela, 14.3 ± 0.98 cm at Latre and 47.0 ± 5.35 cm at Zaragoza). The differences in this variable between plants grown on dry land and irrigated land were greater than those seen for autumn regrowth, probably due to different water availability.

Significant differences ($P < 0.001$) were also seen between accessions in terms of summer regrowth after cutting (5.1 ± 1.41 points on the 1-9 scale at Lagueruela, 6.9 ± 0.68 at Latre, and 6.3 ± 1.03 in Zaragoza).

Table 4 shows the correlation matrix for the studied characteristics. A strong correlation was seen between locations in terms of percentage flowering. Percentage flowering was also significantly ($P < 0.001$) correlated with the capacity of summer regrowth after the first cut, but no significant correlation ($P > 0.05$) was seen with autumn regrowth or growth at the end of winter.

Figure 2 shows the grouping of the accessions based on the evaluated characteristics. Two large groups holding common (cluster 1) or giant (cluster 2) types are evident. These groups can be divided into three subgroups each that discriminate the accessions according to the degree of contamination or crossing (mixed or heterogeneous types) between both major groups. Table 5 gathers the accessions included in each group and subgroup, as well as the means and standard deviations of the measured traits.

Table 2. Means for the measured variables (see Material and Methods) in the 44 sainfoin accessions grown at three locations: Latre (Lat), Lagueruela (Lag) and Zaragoza (Zar)

Accession	% flowering 1st year			Autumn regrowth[1]			Growth (cm) at the end of winter			Summer regrowth[1]		
	Lat	Lag	Zar	Lat	Lag	Zar	Lat	Lag	Zar	Lat	Lag	Zar
1	6.1	24.2	17.9	6.6	5.7	4.8	13.8	12.3	4.8	6.1	3.0	5.1
2	53.8	64.7	83.3	7.0	6.5	5.7	15.2	14.1	5.7	7.2	6.1	7.4
3	45.7	54.7	56.1	6.8	7.0	6.2	14.4	13.5	6.2	7.1	6.0	6.8
4	8.3	32.4	14.3	6.5	6.0	5.9	13.8	13.1	5.9	6.6	3.2	5.0
5	45.9	32.5	30.0	6.8	6.2	6.5	14.7	11.5	6.5	7.2	5.5	7.4
6	54.6	48.5	88.9	7.0	6.6	5.5	14.8	12.2	5.5	7.6	6.3	6.8
7	57.3	42.9	53.7	6.2	6.2	4.5	13.7	11.9	4.5	6.7	4.0	5.7
8	11.1	40.0	20.7	6.8	6.3	6.0	15.4	14.0	6.0	7.2	4.4	7.5
9	65.1	45.4	71.9	7.0	7.4	6.3	15.4	13.3	6.3	7.3	6.5	7.5
10	9.4	64.7	22.2	6.1	6.4	5.2	14.3	12.6	5.2	5.8	3.3	6.0
11	6.4	49.3	51.7	6.5	5.9	6.1	13.8	12.5	6.1	6.3	2.9	4.8
12	27.2	54.1	20.7	6.3	6.4	4.8	14.6	14.3	4.8	7.1	4.5	6.6
13	15.5	39.1	6.7	6.5	6.3	5.0	13.9	12.2	5.0	6.8	3.9	6.5
14	3.0	48.2	13.3	6.5	6.2	6.1	14.6	13.2	6.1	6.4	3.2	5.3
15	23.8	55.3	30.0	6.2	6.3	4.7	13.2	12.7	4.7	7.0	3.9	5.4
16	29.6	32.2	12.5	6.2	5.9	6.1	13.4	12.0	6.1	6.2	4.1	5.3
17	26.0	63.0	29.1	6.9	6.6	6.4	15.4	12.9	6.4	6.5	5.1	7.0
18	48.5	61.0	44.9	6.9	6.7	6.4	15.6	12.0	6.4	7.5	6.4	7.8
19	43.6	37.6	23.3	6.9	6.0	5.2	15.4	11.8	5.2	6.8	4.2	6.0
20	27.2	63.6	7.7	7.1	6.0	6.1	15.2	13.0	6.1	6.1	4.2	3.9
21	61.9	49.7	45.5	6.4	6.5	6.3	15.9	13.2	6.3	7.2	6.1	7.1
22	17.7	42.6	7.5	6.7	6.1	6.3	13.9	13.6	6.3	6.5	2.7	4.8
23	12.1	23.2	0.0	6.4	6.4	6.4	13.1	13.9	6.4	5.9	4.5	4.9
24	80.3	32.8	46.0	7.1	6.4	5.4	15.3	13.4	5.4	6.2	6.0	6.4
25	24.2	67.9	50.0	6.9	6.6	6.6	15.4	13.5	6.6	6.9	6.3	7.3
26	36.1	25.0	0.0	6.2	6.1	5.4	13.2	13.2	5.4	5.3	3.3	4.7
27	48.1	39.4	23.9	6.8	7.0	6.0	16.0	13.1	6.0	6.7	5.5	6.3
28	73.3	39.6	32.4	7.1	6.4	6.1	15.5	14.4	6.1	5.7	5.7	7.0
29	81.1	81.2	81.2	6.7	6.4	5.6	14.2	14.2	5.6	6.5	6.1	7.0
30	73.1	41.8	47.6	6.4	6.2	5.5	14.1	14.1	5.5	7.3	6.2	6.6
31	56.9	46.7	54.5	7.1	6.9	6.8	15.2	14.4	6.8	6.8	5.7	6.9
32	69.3	76.7	82.0	6.8	7.0	5.8	14.5	14.4	5.8	7.1	7.1	6.5
33	66.7	58.3	97.4	6.8	6.9	6.4	15.0	12.8	6.4	7.3	6.5	7.4
34	75.9	65.4	85.0	6.6	7.1	6.0	13.8	14.2	6.0	7.4	6.9	6.8
35	67.8	64.5	75.4	6.8	6.8	6.0	14.6	14.5	6.0	7.6	6.0	6.4
36	85.9	62.2	89.7	6.6	7.0	6.1	15.4	13.6	6.1	7.6	7.7	7.9
37	0.0	15.8	15.0	6.7	6.3	5.8	15.1	14.9	5.8	6.4	3.2	4.0
38	19.5	36.4	31.7	6.3	5.9	5.6	13.0	13.0	5.6	5.5	3.1	5.3
39	63.6	44.5	20.0	6.9	7.0	5.6	13.2	12.3	5.6	7.2	4.6	6.3
40	70.9	47.7	58.3	6.3	7.3	5.6	13.7	12.6	5.6	7.5	7.9	7.2
41	74.2	69.6	100.0	6.4	7.4	6.8	13.2	14.0	6.8	7.5	6.9	7.7
42	48.0	12.4	21.5	5.8	6.3	5.0	11.7	11.7	5.0	6.3	4.6	5.7
43	81.8	67.8	55.6	6.4	7.2	6.0	12.9	14.1	6.0	7.0	6.3	6.6
44	60.1	54.1	58.3	6.3	6.5	5.5	13.0	11.5	5.5	6.4	5.3	5.8
Significance	***	**	***	**	***	*	***	NS	*	***	***	***
LSD 5%	30.75	30.92	13.72	0.62	0.77	1.38	1.82	2.71	1.38	1.05	1.23	1.13

[1] 1 (lowest) to 9 (highest). NS: $P > 0.05$. * $P < 0.05$. *** $P < 0.001$.

Table 3. Significance of the differences detected in analysis of variance involving the studied variables

	% flowered plant 1st year	Autumn regrowth	Growth at the end of winter (cm)	Summer regrowth
Significance Location	*	***	***	***
Significance Accessions	***	***	***	***
Interaction *Location* × *Accession*	***	NS	***	***

NS: P > 0.05. * P < 0.05. *** P < 0.001.

Discussion

To obtain more productive sainfoins, farmers selected more vigorous, taller plants that were able to flower in the sowing year. The differentiation of two types of sainfoin-common (more rustic and unable to flower in the sowing year) and giant (with greater vigour and able to flower in the sowing year) then became possible (Gasparin, 1846).

The capacity to flower in the sowing year has been the main criterion used for classifying accessions as common or giant sainfoin (Michelena and Hycka, 1988; Prosperi *et al.,* 1994). In the present study, the differences between accessions in terms of mean percentage flowering in the sowing year were significant (P < 0.001). However, if this characteristic is considered in isolation it becomes difficult to assign many accessions to either the common or giant group since percentage flowering can be close to 50%, and varied according to the location of the trial. The giant Fakir cultivar (accession number 41), which was used as a control in these trials because of its phenological homogeneity (Prosperi *et al*., 1994), helped in the assessment of the heterogeneity of the accessions. In the irrigated trial, 100% of the plants flowered; however, on the rainfed land only 73% did so. This indicates that water stress can inhibit flowering to a certain extent; dry land conditions are therefore not the most adequate for the classification of accessions as common or giant. The appropriateness of undertaking trials in conditions of no water stress, in order to allow full flowering, has been repeatedly manifested by other authors (Salter *at al*., 1984; Rotili, 1988).

Autumn and summer regrowth, plus winter growth related to vigour and the growth habit have been used in a complementary fashion in the differentiation of the two types of sainfoin by other authors (Michelena and Hycka, 1988; Prosperi *et al.,* 1994). The strong correlation seen between percentage flowering in the sowing year and the capacity of summer regrowth after the cut is one of the characteristics that differentiated the giant sainfoins. This greater regrowth capacity has been reported in other studies (Thonson, 1951, cited by Michelena, 1983; Michelena and Hycka, 1988; Prosperi *et al.*, 1994). In the present study it was sig-

Table 4. Correlation matrix for 12 characteristics measured in Spanish sainfoin grown at Latre (Huesca), Lagueruela (Teruel) and Zaragoza

Trait	1	2	3	4	5	6	7	8	9	10	11	12
1. % flowering plants Latre	—	**	***	NS	NS	***	***	NS	***	NS	NS	***
2. % flowering plants Lagueruela	—	—	***	NS	NS	***	***	NS	***	NS	NS	***
3. % flowering plants Zaragoza	—	—	—	NS	NS	***	***	NS	***	NS	NS	***
4. Autumn regrowth Latre	—	—	—	—	***	***	NS	NS	*	**	**	*
5. Winter growth Latre	—	—	—	—	—	***	NS	NS	NS	*	***	**
6. Summer regrowth Latre	—	—	—	—	—	—	***	NS	***	*	*	***
7. Autumn regrowth Lagueruela	—	—	—	—	—	—	—	*	***	*	NS	***
8. Winter growth Lagueruela	—	—	—	—	—	—	—	—	NS	NS	NS	NS
9. Summer regrowth Lagueruela	—	—	—	—	—	—	—	—	—	*	NS	***
10. Autumn regrowth Zaragoza	—	—	—	—	—	—	—	—	—	—	***	*
11. Winter growth Zaragoza	—	—	—	—	—	—	—	—	—	—	—	NS
12. Summer regrowth Zaragoza	—	—	—	—	—	—	—	—	—	—	—	—

NS: P > 0.05. * P < 0.05. ** P < 0.01. *** P < 0.001.

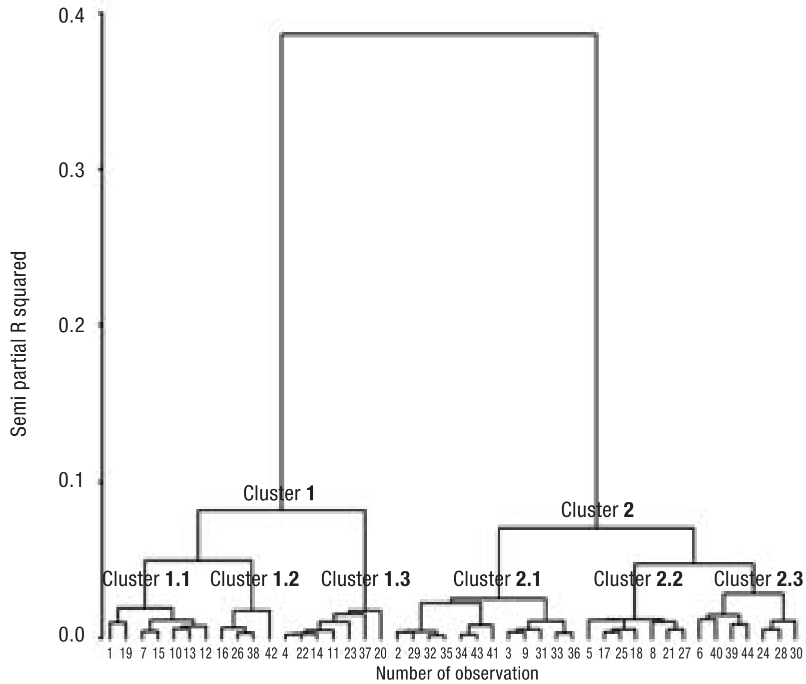

Figure 2. Dendrogram for the 38 sainfoin Spanish accessions and six foreign cultivars.

nificantly different (P < 0.001) at the three locations. Thus, it may be deduced that the capacity of summer regrowth after cutting is also a good indicator of phenological heterogeneity and a classification marker of the common or giant types. This criterion has already been used with other crops such as alfalfa (Delgado *et al.*, 2003). All the above authors agree in that a limited correlation exists between sainfoin type and the amount of winter growth. In the present study no significant (P > 0.05) correlation was observed between percentage flowering in the sowing year, autumn regrowth [except for autumn regrowth in Lagueruela, which was highly (P < 0.001) significant] and growth at the end of the winter.

According to the above descriptions of both types of sainfoin, and after comparing the results obtained with those for the giant cultivar Fakir, accessions 2, 3, 9, 29, 31, 32, 33, 34, 35, 36, 41 and 43 (included in

Table 5. Means and standard deviation (± SD) of the studied variables, distributed according to cluster groups

	% flowering plants	Autumn regrowth	Winter growth	Summer regrowth	Accesion number
Two components					
Cluster 1	26.7 ± 10.22	6.0 ± 0.25	24.2 ± 2.40	5.1 ± 0.47	
Cluster 2	57.9 ± 15.27	6.5 ± 0.22	25.4 ± 1.76	6.8 ± 0.44	
Six components					
Cluster 1.1	32.2 ± 11.48	5.8 ± 0.14	24.0 ± 0.87	5.5 ± 0.39	1,7,10,12,13,15,19
Cluster 1.2	25.4 ± 3.82	5.9 ± 0.15	21.4 ± 3.27	4.8 ± 0.42	16,26,38,42
Cluster 1.3	21.9 ± 9.70	6.3 ± 0.11	25.9 ± 1.22	4.8 ± 0.28	4,11,14,20,22,23,37
Cluster 2.1	69.8 ± 10.15	6.6 ± 0.21	25.5 ± 1.13	7.0 ± 0.30	2,3,9,29,31,32,33,34, 35,36,41,43
Cluster 2.2	41.1 ± 10.13	6.6 ± 0.13	26.5 ± 0.85	6.7 ± 0.41	5,8,17,18,21,25,27
Cluster 2.3	54.1 ± 7.03	6.3 ± 0.19	23.9 ± 2.40	6.6 ± 0.56	6,24,28,30,39,40,44

Cluster 2.1), with the greatest growth all, fall within the giant sainfoin group. These accessions came mainly from the provinces of Burgos, Logroño, Soria and Huesca. Accessions 4, 11, 14, 20, 22, 23 and 37 (included in Cluster 1.3) are the closest to a typically common sainfoin type. These accessions came mainly from the provinces of Teruel, Castellón, Guadalajara, Lleida and Palencia. However, all provinces were home to accessions with a certain degree of phenological heterogeneity; these grouped into different clusters (1.1, 1.2, 2.2 and 2.3). This heterogeneity may be attributed to seed importation from other countries (Pujol, 1974) and the free seed market among farmers from different areas (Michelena, 1983; Delgado *et al*., 2002).

Despite the indiscriminate marketing of seed, the present results show that common and giant sainfoin types can still be differentiated. Two easily identified characteristics —percentage flowering in the sowing year and the speed of regrowth after the spring cut— contribute to the classification of these plants into one type or the other, and could facilitate the selection of cultivars.

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