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Study of the leaf anatomy of the Iberian species of genus *Anthoxanthum* (*Poaceae*)

Abstract

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The taxonomic importance of leaf anatomy in the *Poaceae* has been highlighted by many authors and applied to a great number of genera belonging to this family. This study provides new information which may help clarify the relationships among the different taxa of the genus *Anthoxanthum* present in the Iberian Peninsula and to evaluate the influence of the environment on the anatomical characteristics to analyze their validity for taxonomic purposes.
The anatomical characteristics of 110 samples from 22 populations of the five Iberian taxa of *Anthoxanthum* were analyzed. Of each sample 12 characteristics of the epidermal surface and 10 characteristics of the leaf transverse section were measured and all information was stored in a database. The similarity among samples was determined using the Phi Coefficient The relationships among samples were summarised in a phenogram carried out using Clustering Methods. The influence of environmental parameters on the anatomical characteristics was determined by a Canonical Correspondence Analysis.

Introduction

The genus *Anthoxanthum* s. str. (*Poaceae*; *Pooideae*) comprises around 15 species distributed in Europe, Asia and Africa, mainly in middle latitudes, but also in lower ones reaching South Africa and Sumatra (Nicora & Rugolo 1985). Moreover, the species *Anthoxanthum odoratum* has been introduced in America and Australia, where locally it constitutes an ecological problem (Gould & Shaw 1992).

In Europe there are seven species of *Anthoxanthum* (Tutin & al. 1980), four of which are present in the Iberian Peninsula: *Anthoxanthum aristatum* and *Anthoxanthum ovatum*, annual and diploid species, *Anthoxanthum odoratum*, a perennial species that constitutes a polyploid complex and *Anthoxanthum amarum* Brot., a perennial and polyploid species endemic from North-West Iberian Peninsula.

Although many authors consider that leaf anatomy is a very important tool in grass ta-xonomy (Ellis 1976, 1979; Devesa 1992), its validity has been pointed out as doubtful because of the influence of the environment on these characteristics. (Aiken & al. 1985).

In this work we have analyzed the foliar anatomy of the four Iberian species of *Anthoxanthum*. We have also established their anatomical relationships and calibrated the taxonomical usefulness of these data in this genus.

Materials and methods

In the present study 110 samples from 22 populations of *Anthoxanthum* were analyzed (six populations for *A. amarum*, seven for *A. odoratum* and *A. aristatum* and two for *A. ovatum*). For each sample, the abaxial epidermal surface and the leaf transverse section were analyzed using fragments of the lower third of the basal leaf that were taken from dried specimens. The characteristics studied were those pointed out by many authors as taxonomically interesting (Ellis 1976, 1979; Devesa 1992).

To get suitable samples for microscope observation the method of Devesa (1992) was used. For a more detailed study the technique applied was a double staining, using alcian blue and safranine following Ruiz & al. (1985). The terminology employed was that from Ellis (1976, 1979) and Devesa (1992).

The obtained data were stored in a database, afterwards a dendrogram was performed using the program SPSS 10.0. The distances measure used was the "Phi" Coefficient, and the grouping method was the UPGMA (Unweighted pair-group method, arithmetic average).

Finally, to calibrate the influence of the environment in the anatomical characters a Canonical Correspondence Analysis was performed using the CANOCO program 4.0 (Ter Braak & Smilauer 1998).

The environmental parameters included in the analysis were: soil pH, annual mean temperature, mean temperature of the hottest and the coldest months, annual rainfalls and existence of a drought period during the year.

Results and discussion

Anatomical description of the taxa

A low degree of variation was observed among the different taxa regarding to the leaf transverse section, for this reason, only the epidermal surface data were used for the statistical analysis.

Based on our data, the transverse section can be described for the genus as follows: Epidermal cells roundish and unequal. The adaxial surface presents furrows and ribs more or less conspicuous. Bulliform cells are nearly always present, lacking only in a few individuals of *Anthoxanthum amarum* and *Anthoxanthum odoratum*. Bulliform cells are forming fan-shaped groups of 4-7, specially in furrows of the adaxial surface.

The clorenchyma is not radiate. Vascular bundles (8-21 for each leaf) are not angular in outline. They are mostly free, but some of them (those of first order) are joined to the epidermal surface by strands of sclerenchyma. Generally they are joined only to the abaxial surface. The vascular bundle sheaths are double and the external is interrupted except in the main vascular bundle, where it is complete.

Our results are quite consistent with those given by other authors, e.g. Metcalfe (1960) and Devesa (1992). We have observed differences only in the presence of bulliform cells and in the type of vascular bundle sheaths.

For the epidermal surface the results for each taxon are summarised in Table 1. *Anthoxanthum ovatum* can be distinguished by the typology of the intercostal zones. *Anthoxanthum odoratum* presents the highest variability and it is characterised by the

absence of short cells both in the intercostal and in the costal zones. *Anthoxanthum amarum* is similar to *A. odoratum* except for the presence of siliceous short cells. *Anthoxanthum aristatum* is defined by the size of its long cells. Inside this taxa two varieties were analyzed, but no significant differences between them have been observed.

STATISTICAL RESULTS

As can be seen in Figure 1 the anatomical data from the epidermal surface of the leaf are quite suitable to separate the different taxa present in the Iberian Peninsula. Specially important characteristics are the typology and length of long cells in costal zones and the presence of short cells in the intercostal ones.

A. amarum and *A. odoratum* are the best characterised taxa. It is worth noting that in

Table 1. Abaxial epidermal surface of Genus *Anthoxanthum* L. IZ.- Intercostal zones; CZ.- Costal zones. CB.- Bulliform cells. Between brackets are those data less representative. *Epidermal typology*: H, homogeneous (without neither short cells nor exodermic elements); HE, homogeneous-exodermic (without short cells, but with exodermic elements); SE, siliceous-exodermic (with short cells and exodermic elements). *Long cells*: l2, cells three times longer than width and with thin walls; l3, cells three times longer than width and with thickened and undulating walls. *Short cells*: S1′, isodiametric siliceous cells; *Exodermic elements*: P2, hooked prickel hairs; P, macro-hairs. *Stomata*: P/p, Paracitic stomata with parallel-sided subsidiary cells; P/p-d, Paracitic stomata with parallel-sided or dome-shaped subsidiary cells.

Taxa	Epidermal typology		Long cells			
	IZ	CZ	IZ	Mean size	CZ	Mean size
A. amarum	HE/SE	SE	LE/L2	550•43 µm	L3	225•34 µm
A. odoratum	S/SE	SE	L2	242•20 µm	L2	100•13 µm
A. ovatum	HE/(SE)	HE	L3/(L2)	500•34 µm	L2/(L3)	198•14 µm
A. a. var. *aristatum*	H/(HE)	SE	L2/(L3)	234•23 µm	L2	120•12 µm
A. a. var. *welwitschii*	HE	SE	L2	238•32 µm	L2	127•15 µm

Taxa	Short cells				Exodermic elements			
	IZ	Mean size	CZ	Mean size	IZ	Mean size	CZ	Mean size
A. amarum	S1'	36•45 µm	S1'	25•36 µm	P2	48 µm	P2/P	65 µm
A. odoratum	S1'	33•23 µm	S1'	10•7.5 µm	(P2)	35 µm	P2	42 µm
A. ovatum	-	-	-	-	P2/(P)	56 µm	P2/P	74 µm
A. a. var. *aristatum*	-	-	S1'	31°•18 µm	(P2)	55 µm	P2/(P)	62 µm
A. a. var. *welwitschii*	-	-	S1'	28•31 µm	P/(P2)	49 µm	P/(P2)	55 µm

Taxa	Stomata		Bulliform cells
	Type	Mean size	CB
A. amarum	P/p	75•45 µm	No
A. odoratum	P/p	46•23 µm	Yes
A. ovatum	P/p-d	60•29 µm	No
A. a. var. *aristatum*	P/p	49•22 µm	Yes
A. a. var. *welwitschii*	P/p	44•20 µm	Yes

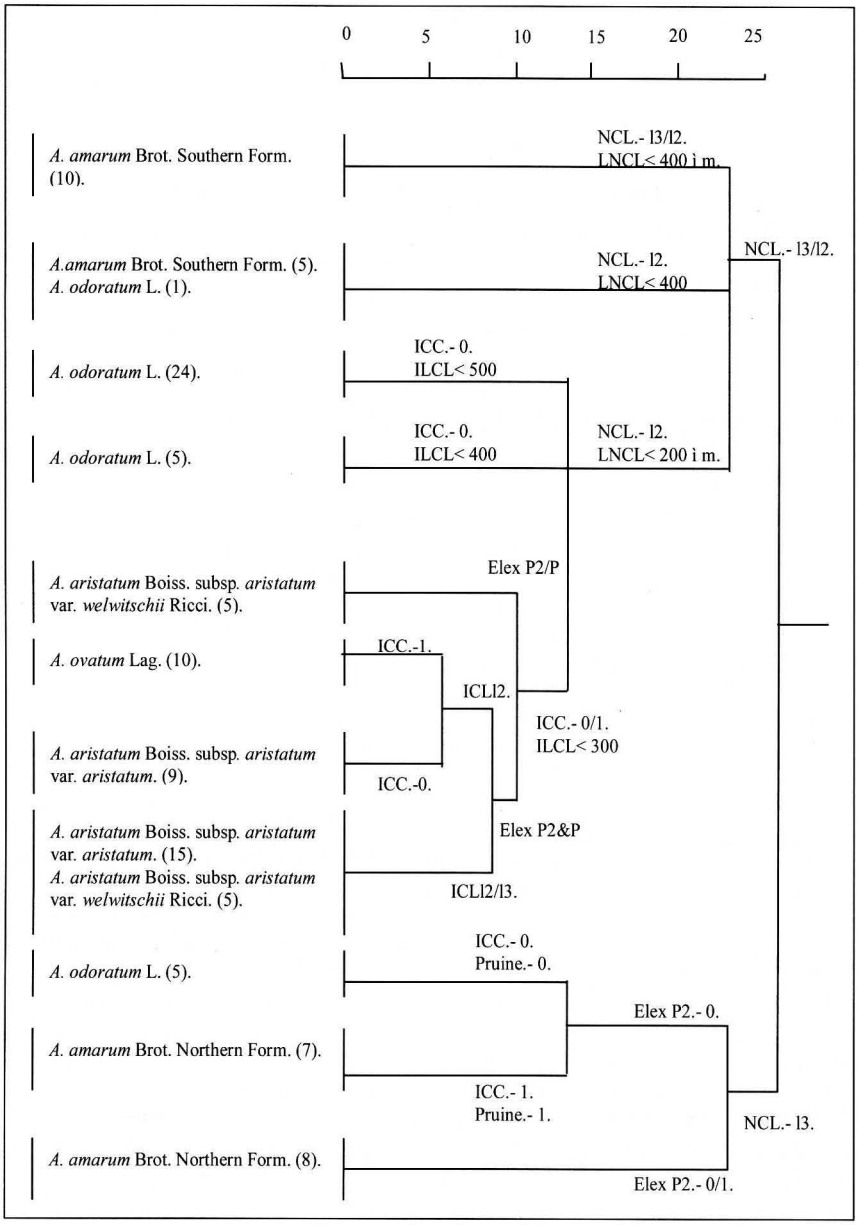

Fig. 1. Dendrogram obtained with anatomical data. NCL.- Typology of the long cells in the costal zones (l2, l3 or both). LNCL.- Length of the long cells in costal zones. ICC.- Presence or absence of short cells (0, absence; 1, presence). ILCL.- Length of the long cells in the intercostal zones. ICL.- Typology of the long cells in intercostal zones (l2, l3 or both). Elex P2.- Presence of hooked prickle hairs in costal zones (0, absence; 1, presence). Elex P2/P.- Presence of hooked prickle hairs or macro-hairs in costal zones. Elex P2&P.- Presence of both hooked prickle hairs and macro-hairs in costal zones.

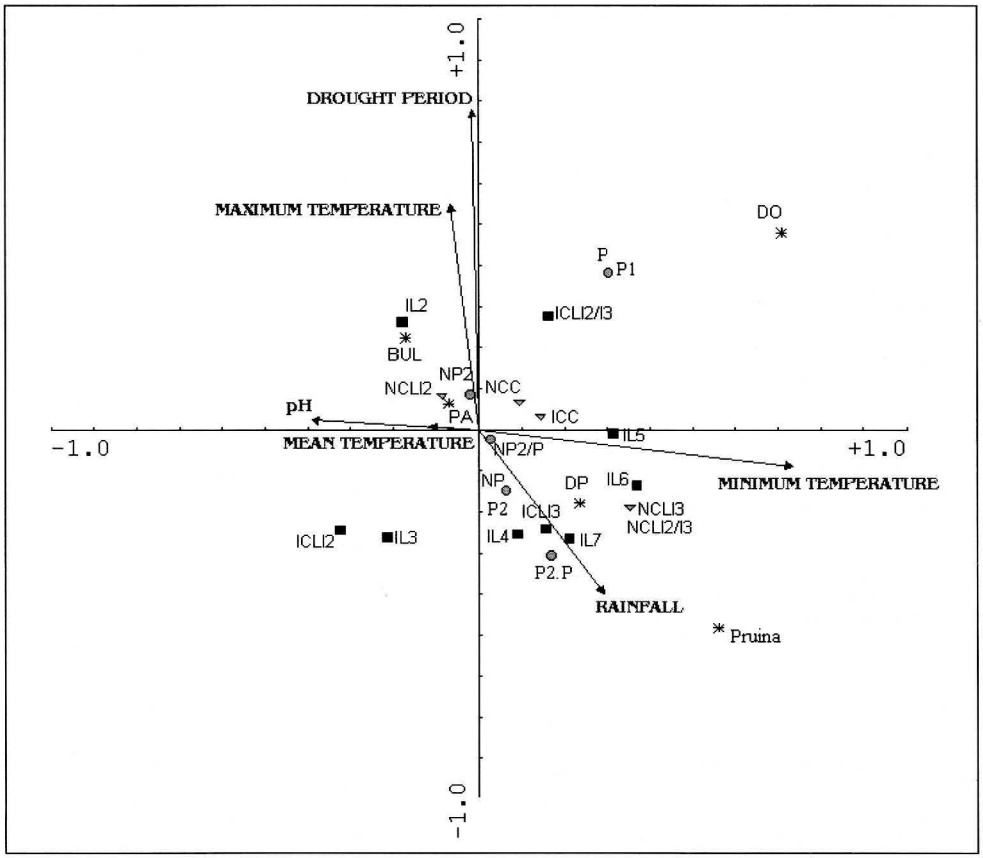

Fig. 2. Graphic results of the Canonical Correspondence. Arrows are referred to the enviromental parameters. Pruine.- Presence of pruine on the leaf surface. BUL.- Presence of Bulliform cells. DO.- Stomata with dome-shaped subsidiary cells. PA.- Stomata with parallel-sided subsidiary cells. DP.- Stomata with both types of subsidiary cells. P.- Presence of macro-hairs in the intercostal zones. P2.- Presence of hooked prickle hairs in the intercostal zones. P2.P.- Presence of both types of exodermic elements in the intercostal zones. P1.- Presence of no-hooked prickle hairs. NP.- Presence of macro-hairs in the costal zones. NP2.- Presence of hooked prickle hairs in the costal zones. NP2/P.- Presence of both exodermic elements in the costal zones. NCC.- Presence of short cells in the costal zones. ICC.- Presence of short cells in the intercostal zones. ICLl2.- Long intercostal cells l2. ICLl3.- Long intercostal cells l3. ICLl2/l2.- Long intercostal cells l2 or l3. NCLl2.- Long costal cells l2. NCLl3.- Long costal cells l3. NCLl2/l2.- Long costal cells l2 or l3. IL.- Length of the intercostal long cells (2.- 100-200 μm; 3.- 200-300 μm; 4.- 300-400 μm; 5.- 400-500 μm; 6.- 500-600 μm; 7.- 600-700 μm).

A. amarum two groups (a southern and a northern group) are clearly separated by the presence of cells three times longer than width with smooth walls (l2) in costal zones.

A. aristatum and *A. ovatum* were very mixed in the dendrogram, and they are separated only at low levels by the presence of short cells in the intercostal zones. The results obtained in the dendrogram do not support the existence of two varieties in *A. aristatum* subsp. *aristatum*.

To calibrate the usefulness of the anatomical data, the influence of the environment over them has been studied with a Canonical Correspondence Analysis. The results show a strong correlation between the studied characteristics and the environmental parameters (Fig. 2). Fifty-nine percent of the variability in the characteristics is explained by the environment (Table 2). Although a direct correlation can be seen in some cases, for example between the annual rainfall and the type of exodermic elements in costal zones, in most cases is a combination of the different environmental parameters which affects the anatomical characteristics.

Table 2. Summary of the results of the Canonical Correspondence Analysis.

Axes	1	2
Correlation between the environmental parameters and the characteristics	0,892	0,701
Eigenvalues	0,315	0,253
% of the variation explained by the relationship between the characteristics and the environment (accumulative)	**33,9**	**61,1**
Sum of all the eigenvalues	3,296	
Sum of all the canonical eigenvalues	0,931	
Montecarlo test		
p-value	0,005	

Conclusions

1. The anatomical leaf characteristics are suitable to distinguish the Iberian *Anthoxanthum* taxa., but it is necessary to consider that the high influence of the environment on the epidermal surface characters makes these data less reliable.
2. In *A. aristatum* Boiss. subsp. *aristatum* the separation between two varieties is not supported by this anatomical study.
3. This is the first time that *Anthoxanthum amarum* leaf anatomy has been studied. In this species we have observed the existence of two well-differentiated ecotypes. A molecular analysis would be necessary to establish their taxonomical status.

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