Journal of the American Pomological Society 60(1):37-45 2006

Identification and Morphological Description of Cultivars of Chestnut (*Castanea sativa* Mill) of the Region of Verín-Monterrei (Ourense, Spain)

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Abstract

In this study we classified morphologically the most widespread cultivars of chestnut grown in the region of Verín-Monterrei: 'Bermella', 'Blanca', 'Boullona', 'Calva', 'Casarella', 'Corronchuda' 'das Vinas', 'Famosa', 'Foleiro', 'Inxerta', 'Longal', 'Monfortina', 'Soutiña', 'Touro' and 'Vilamaesa'. Based on biometrical analysis of leaves, flowers and nuts, most of variability among cultivars was explained by variations in leaf and nut morphology.

Introduction

At least 143 different cultivars of chestnuts have been identified in Galicia (northwest Spain) (9, 14). The classification of different cultivars is not easy because different synonyms are used in different regions for referring to the same cultivar. It may also occur that the same name is used for different cultivars. In Galicia, the suitable places for the production of chestnut are usually on mountain slopes, in acid soils that are at least 70 cm deep. These characteristics are found in the region of Verín-Monterrei, where chestnuts are very important for industries involved in processing. The objective of our work was to characterize cultivars of chestnut from the region of Verín-Mnterrei (Ourense, Spain) on the basis of morphological measurement of leaves, flowers and nuts, and to identify morphological traits that play an important role in determining the differences among cultivars, using a multifactorial method.

Materials and Methods

In 1997 and 1994 a series of meetings with producers from the region of Verín-Monterrei (SE of Ourense, Spain) took place to gather information on aspects related to the most common cultivars in the region, names of the cultivars, size, production, suitability for conservation, diseases and pests and any further information of interest.

Material

Nine areas of production were selected in different parts of the region. Based on information provided by the producers, we chose 80 trees, of 15 cultivars for our study. 'Bermella', 'Blanca', 'Boullona', 'Calva', 'Casarella', 'Corronchuda' 'das Vinas', 'Famosa', 'Foleiro', 'Inxerta', 'Longal', 'Monfortina', 'Soutiña', 'Touro' and 'Vilamaesa'.

A variable number of trees of each cultivar was selected according to its relative distribution in the region. Of the least widespread cultivars (for example 'Boullona' or 'Foleiro') we selected samples from 3 trees; while of the most widespread cultivars (for example 'Famosa' or 'Longal'), we sampled up to 10 trees for cultivar. Samples were taken at three different times during 1998, coinciding with the times when the leaves, flowers and fruits were completely developed.

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Table 1. Measures and standard deviations of biometrical parameters of leaves and flowers.

Cultivar	Length (cm)	Width (cm)	L/W	Teeth	Veins	Petiole (cm)	Basal angle	Length of catkin (cm)	Length of stamen
Bermella	15.9±3.0	5.4±0.9	2.8±0.1	18±3	21±4	1.6±0.5	76.9±19.6	17.3±2.8	Mesostaminated
Blanca	16.2±2.3	6.9±1.0	2.3±0.1	16±3	18±3	1.5±0.4	76.4±14.5	19.6±1.9	Astaminated and longistaminated
Boullona	18.8±4.2	5.9±1.1	2.9±0.0	15±3	18±3	1.9±0.3	63.6±14.4	20.1±2.4	Mesostaminated
Calva	15.4±2.5	6.0±3.0	2.5±0.1	13±3	16±2	1.7±0.5	76.8±17.3	21.0±2.2	Mesostaminated
Casarella	14.2±2.2	5.3±0.8	2.6±0.1	16±3	18±2	1.6±0.4	87.6±15.9	16.8±2.3	Longistaminated
Corronchuda	17.1±3.1	5.7±0.1	2.9±0.0	17±3	19±2	1.7±0.3	91.3±18.0	19.3±3.7	Mesostaminated
Das Viñas	15.2±2.8	6.0±1.2	2.5±0.1	16±2	20±3	1.4±0.3	81.4±14.8	21.8±2.8	Mesostaminated
Famosa	15.0±2.6	5.4±0.9	2.7±0.1	16±3	18±6	1.7±0.5	80.7±16.4	22.4±3.2	Brachistaminated
Foleiro	14.2±1.7	6.2±0.0	2.3±0.1	14±2	16±2	1.4±0.4	85.7±14.1	18.1±2.3	Mesostaminated
Inxerta	16.2±3.2	6.1±1.3	2.6±0.1	15±3	19±2	1.6±0.4	68.8±18.4	19.0±3.9	Longistaminated
Longal	15.4±3.4	5.8±1.2	2.6±0.1	14±3	17±3	1.9±0.7	77.9±13.2	17.4±3.1	Brachistaminated
Monfortina	17.3±3.8	7.0±1.5	2.4±0.1	18±4	20±3	1.8±0.4	82.5±18.0	20.6±5.0	Longistaminated
Soutiña	15.5±2.7	6.4±0.9	2.4±0.1	14±3	17±2	1.6±0.1	79.6±29.7	13.7±1.5	Brachistaminated
Touro	20.0±4.9	5.8±0.9	2.6±0.1	13±2	16±2	2.2±0.7	72.2±17.4	18.8±2.6	Longistaminated
Vilamaesa	16.0±2.4	6.5±1.0	2.4±0.1	16±3	19±3	1.6±0.5	80.3±12.6	18.4±2.8	Brachistaminated

L/W: relation length/width of the leaf.

*According to the classification proposed by Pereira and Fernández (1997a) and UPOV (1988).

Biometrical methods

Leaves. Following the recommendations by Pimentel-Pereira et al. (19) and UPOV (22), we selected 60 leaves from each tree for measuring those parameters considered suitable by previous researchers for differentiating cultivars: length or height, width, ratio of height/width, leaf shape, number of teeth and veins in both sides of the leaf, location of the first tooth, length of the petiole and basal angle of both sides of the leaf (4, 13, 15, 19, 20, 21).

Flowers. (25 male catkins) were picked following the protocol proposed by Bencat and Bolvansky (6). They underwent the measurements recommended by different authors for cultivar differentiation: length of catkins and stamens and number of glomeruli (2, 4, 6, 15).

Chestnuts. Following the method proposed by Guerreiro (10), and studied and completed by Pimentel-Pereira and Torres-Pereira (20), we sampled 100 nuts from the 80 trees that had been selected. From these 100 chestnuts we selected 25 at random for biometrical and morphological analysis based on: thickness, width, height, ratio of width/height, length of style and stigma, shape of the fruit, shape of the hilum, unit weight, volume of 100 nuts, density and color of the outer shell of chestnuts (1, 2, 4, 5, 8, 13, 15, 16, 18, 20, 22). Color measurements followed the parameters of the Lab chromatic scale (L*, a* and b*) and from them we assessed tone (H*), chroma (*C) and saturation (S*).

Statistical analysis.

We analyzed the data in four steps, using the statistical program SPSS 9.0 for Windows. First we carried out data analysis, and then we assessed the significance of each variable. Afterwards, we assessed the correlation matrix beforehand, and we applied a factorial analysis (using the method of the principal components), only conserving those with values higher than one. Next we used varimax rotation to facilitate the interpretation of factors, on the basis of their factorial scores. Finally, we grouped cultivars according to similarity, taking into account the main components obtained in the previous step, obtaining a dendogram or logic chart. We used the hierarchical conglomerate using intergroup relationship, the square euclidian distance (as a measure of the proximity among intervals) and standardizing by means of Z scores for variables.

Results and Discussion

Leaves. (Table 1) All cultivars had "wide" leaves ($\geq$ 5 cm) and none of them had short leaves ($\leq$ 13 cm). The number of veins was always higher than the number of teeth and in all cases, the first tooth was in the basal position, in accordance with the results published by Pereira and Fernández (15).

Flowers. (Table 1). Most of the cultivars had mesostaminate catkins (with length of stamens between 2.6 and 4.5 mm). Our data do not support the conclusion of Bencat and Bolvansky (6) according to whom "short catkins with stamens are shorter and thinner and long catkins with stamens are long and thick." The number of glomerules varied between 56 and 156, and so we assessed the mean for this variable.

Fruits. (Table 2). Six of the 15 cultivars studied ('Casarella', 'das Viñas', 'Foleiro', 'Longal', 'Monfortina' and 'Vilamaesa') had "thin" chestnuts (<1.8 cm) and none had "wide" nuts ($\geq$ 3.5 cm). The cultivars 'Inxerta', 'Longal' and 'Bermella' had the longest fruits in accordance with the results found by Pereira and Fernández (15); styles and stigmas were generally "long" ($\geq$ 0.95 cm). We have not found any cultivar that weighed more than 13g/nut and all, except 'Boullona', had low density ($\leq$ 1.02 g/ml). As regards nut color, the brightest tones were found in 'Inxerta', 'Calva', 'Foleiro', 'Soutiña' and 'das Viñas', while the least bright were found in 'Casarella', 'Blanca', 'Monfortina', 'Ber-

Table 2. Means and standard deviations of biometrical parameters of fruits.

Cultivar	Width (cm)	Length (cm)	Thickness (cm)	W/H x 100	Style + stigma (cm)	Style (g)	Unit weight (ml)	Volume (g/ml)	Density
Bermella	3.2±0.3	2.9±0.2	1.9±0.3	113±9.7	1.0±0.1	0.7±0.1	10.0±2.4	1242±281	0.9±0.4
Blanca	3.0±0.2	2.7±0.2	1.8±0.0	112±7.5	0.9±0.1	0.6±0.1	8.5±1.6	1550±638	0.5±0.1
Boullona	3.1±0.2	2.6±0.2	2.1±0.2	120±8.3	0.9±0.1	0.6±0.1	10.6±2.1	1133±210	1.0±0.0
Calva	3.2±0.4	2.8±0.2	1.8±0.3	111±13	0.7±0.1	0.7±0.1	9.5±2.5	993±179	1.0±0.3
Casarella	2.6±0.2	2.5±0.4	1.6±0.2	104±15	1.0±0.2	0.6±0.1	4.9±1.6	756±214	0.5±0.3
Corronchuda	3.1±0.3	2.7±0.2	1.9±0.3	116±9.5	0.5±0.1	0.6±1.2	8.8±2.2	980±28	0.9±0.4
Das Viñas	3.1±0.3	2.7±0.2	1.7±0.3	112±6.3	1.1±0.1	0.7±0.1	8.6±2.9	1025±287	0.9±0.3
Famosa	3.1±0.2	2.7±0.2	1.8±0.3	114±10.5	1.0±0.1	0.6±0.1	8.6±1.8	1110±353	0.8±1.1
Foleiro	2.8±0.2	2.5±0.2	1.8±0.2	113±5.7	1.1±0.1	0.7±0.1	7.4±1.9	1300±424	0.4±0.0
Inxerta	3.2±0.2	3.0±1.8	1.8±0.2	111±12.4	1.0±0.1	0.7±0.1	9.5±2.2	1230±119	0.7±0.3
Longal	2.6±0.3	2.9±0.2	1.6±0.2	91±12.9	1.2±0.1	0.8±0.1	6.6±1.3	1085±442	0.6±0.2
Monfortina	2.8±0.4	2.6±0.3	1.7±0.3	110±10.8	1.0±0.1	0.6±0.1	7.2±3.0	900±141	0.7±0.5
Soutiña	3.0±0.2	2.8±0.2	1.8±0.3	126±9.9	1.1±0.1	0.7±0.1	8.0±1.6	1400±565	0.6±0.1
Touro	3.2±0.3	2.66±0.2	2.1±0.3	110±9.4	0.9±0.1	0.5±0.1	10.4±2.7	1150±353	1.0±0.4
Vilamaesa	3.0±0.3	2.7±0.2	1.6±0.2	111±6.8	1.0±0.1	0.7±0.1	7.1±2.4	967±265	0.5±0.1

W/H 100: relation width/height of the fruit multiplied by 100

mella' and 'Corronchuda'. We observed very low values in the rates of red-green color (a*) and blue-yellow (b*); however b* was higher than a* in absolute value in the cultivars 'Famosa', 'Foleiro', 'Inxerta', 'Longal', 'Soutiña' and 'Vilamaesa', so in these cases the yellow component was predominant over red. The nuts of 'Monfortina' and 'Touro' ware the darkest as they presented higher saturation values (S*).

The different observations reported by other studies on Galician cultivars (15, 16) were attributed to phenotypic and/or genotype variations, since they also exist among trees belonging to the same cultivar which come from different areas (17).

Significance of the variables studies.

In the case of variables related to bilateral symmetry of leaves (number of teeth, veins and basal angle), the statistical treatment was carried out only with the values corresponding to the left side of the leaf, after checking that there were not significant differences between values for the two sides.

In the case of leaves we checked that all the quantitative parameters assessed were significant for identifying these cultivars, agreeing with the results found in the bibliography (1, 4, 12, 13, 15, 18, 19, 20, 21). Our results are in accordance with a previous study done on some of these same cultivars (12), in which it was concluded that measures of height and width of the leaf, number of teeth and veins, were suitable for differentiating cultivars.

Among the parameters assessed in flowers, only catkin length proved to be suitable for differentiation of cultivars, as found by other investigators (2, 4, 6, 15).

In fruits we observed that, except in the volume and density of chestnut, the rest of the parameters are significant for differentiating cultivars, in accordance with the results obtained by other authors (1, 2, 4, 5, 8, 11, 13, 15, 16, 18, 20).

Correlation matrix.

After assessing the correlation matrix between the 21 quantitative variables that proved to be significant in the previous step, we observed a high correlation among them, as is also shown in the low value of their determinant.

When we obtained factors using the method of principal components we found that the 21 biometrical and chromatic variables were saturated in 6 main components with self values higher than one, which accounts for approximately 85% of total variance (Table 4). Those variables related to the morphology of fruits and color, mainly intervene in the 1st, 2nd and 4th components and account for 53.7% of the variance among cultivars. The 3rd and 5th components are mainly influenced by parameters of leaves and represent the second source of variance among cultivars, accounting for 23.8% of it (and 77.5% of the accumulated variance). The last factor is related to catkin length, accounting for 7.6% of the variance, with which 85% of total variance is finally justified.

As we have biometrical data of leaves, flowers and fruits of some cultivars picked up during the harvest of 1994, they underwent the same statistical analysis (factorial analysis) to establish what variables accounted for the main part of the differences among those cultivars in that harvest. We found that 78.3% of total variance could also be attributed to parameters related to the morphology of fruits and leaves.

This led us to conclude that those parameters related to the morphology of chestnuts and leaves allow for differentiation of cultivars of chestnuts belonging to the same harvest year. This does not mean that for the same cultivar, there are no differences between values for different harvest years. In fact, when we compared the means of the 4 variables with more influence on fruit morphology in both years, we observed (Table 5)

Table 3. Means and standard deviations of the chromatic parameters of fruits.

Cultivar	L*	a*	b*	Hue*	C*	S*
Bermella	72.8±21.5	6.9±6.9	4.6±4.8	50.5±22.2	10.3±6.2	12.5±13.8
Blanca	70.0±20.6	6.0±7.7	4.1±5.6	43.6±34.4	10.4±5.8	12.7±25.4
Boullona	94.3±16.6	-5.3±5.0	0.3±6.3	24.1±59.6	8.0±5.2	17.7±15.0
Calva	99.7±7.7	-4.5±6.8	-0.4±7.1	34.4±37.8	9.3±5.6	17.7±15.8
Casarella	59.3±6.3	8.0±3.2	5.2±2.0	56.2±6.3	9.6±3.7	7.3±3.6
Corronchuda	73.4±24.5	3.0±8.6	2.9±4.9	50.0±35.0	9.7±4.7	11.7±12.1
Das Viñas	97.2±12.6	-4.8±5.9	1.2±5.4	28.7±45.3	10.6±6.1	20.3±20.3
Famosa	89.1±22.0	3.8±8.2	6.1±7.6	33.2±37.8	10.7±7.8	14.4±14.2
Foleiro	95.4±15.3	3.2±6.7	4.6±6.6	33.2±32.1	9.4±5.5	14.9±11.3
Inxerta	100.0±7.8	0.3±5.8	4.0±5.6	43.0±53.3	7.2±5.4	21.7±16.4
Longal	93.8±10.2	0.3±7.9	1.2±3.7	25.2±38.4	5.1±7.2	35.4±40.3
Monfortina	70.6±17.1	7.5±6.0	4.9±4.1	54.5±9.7	10.1±5.5	74.6±25.3
Soutiña	98.5±7.0	2.6±6.6	7.5±6.9	24.7±36.1	10.4±6.7	14.5±11.1
Touro	94.0±13.6	-3.1±5.7	-0.4±3.4	31.0±50.0	5.9±4.3	61.8±9.9
Vilamaesa	90.2±16.1	3.7±9.0	3.9±3.9	27.0±48.7	7.7±8.1	20.6±15.7

Table 4. Main components in the multifactorial analysis.

Variables	Components (% variance)					
	1 (22.3%)	2 (18.6%)	3 (13.7%)	4 (12.8%)	5 (10.1%)	6 (7.6%)
Fruit width	0.786	0.409	0.172	-0.157	-1.479E-02	0.152
Fruit height	-0.181	0.659	0.368	-0.103	-0.199	-0.155
Fruit thickness	0.899	0.201	-0.106	0.213	-9.416E-02	-5.665E-04
h/l*100	0.661	-0.299	0.303	-7.274E-02	0.422	0.236
Style+stigma	-0.784	0.412	-3.275E-02	-0.125	8.020E-02	-0.109
Style	-0.805	3.715E-02	0.321	8.261E-02	0.103	-0.215
Unit weight	0.701	0.513	0.179	9.720E-02	-0.258	0.197
Leaf width	1.862E-02	3.942E-02	8.183E-02	4.492E-02	0.942	-7.360E-02
Leaf length	0.545	4.944E-02	0.556	0.391	0.125	-5.838E-02
l/h leaf	-0.427	3.370E-02	-0.279	-0.283	0.727	4.882E-02
l.petiole	0.288	1.695E-02	-0.151	0.883	-0.188	-1.313E-02
D.left	6.493E-02	-0.197	0.883	-0.232	-6.969E-02	6.726E-02
N.left	-8.032E-02	-0.138	0.943	-0.124	2.444E-03	7.513E-02
A.left	0.519	0.689	0.114	0.263	-3.245E-02	-6.489E-02
l. catkins	0.196	3.829E-03	0.185	-3.720E-02	6.627E-02	0.877
L*	3.336E-02	0.887	-0.283	2.229E-02	0.133	0.108
a*	-0.286	-0.712	0.248	-0.199	0.128	-0.440
b*	-0.210	-0.362	0.201	-0.460	0.229	-0.587
Hue*	2.833E-02	-0.782	0.327	-9.515E-02	-8.640E-02	-2.382E-02
C*	0.178	-0.394	0.231	-0.769	0.171	7.233E-02
S*	-3.320E-02	-0.113	1.260E-02	0.748	0.465	0.226

Table 5. Comparison of some variables related to fruit morphology, corresponding to the harvest of 1994 and 1997.

	Width	Height	Thickness	Unit weight
Means for the harvest of 1994	3.26	3.24	2.03	12.68
Means for the harvest of 1997	3.05	2.76	1.81	8.61
Variation with respect to the harvest of 1994 (%)	−6.44	−14.82	−10.84	−32.10

that those chestnuts from the harvest of 1994 were remarkably bigger. These variations which affect some morphological parameters of the nut were also observed in several publications by Italian investigators (1, 3, 7), where they concluded that the weight of fresh chestnut and nut width, are "traits highly influenced by environmental conditions".

When we analyzed intracultivar variations for the different cultivars in both harvests, we found that the chestnuts of 'Vilamaesa' showed more variation in unit weight (53.8%) in width (14.1%) and thickness (20.97%) (Table 6). However, we observed little variation in the weight of nuts from 'Touro' between both harvests (5%), and we observed little variation in width, height and weight in the case of 'Bermella'.

Cluster analysis. Dendogram. In this step we wanted to group the different cultivars into conglomerates according to their degree of similarity. So, we selected as relevant parameters the 6 principal components which accounted for 85% of total variance. We chose intergroup relationship as the method of grouping and the square euclidian distance as the measure for establishing proximity between variables, obtaining the dendogram presented in Figure 1. Four groups of variables can be distinguished which, in turn, are part of a bigger conglomerate. The first group is formed by a small cluster, which includes the 5 cultivars 'Inxerta', 'Vilamaesa', 'Blanca', 'Foleiro' and 'Soutiña'. The second conglomerate is formed by a cluster of 'Corronchuda', 'Famosa', 'Bermella' and 'Casarella'. A third cluster is attached to these two groups, formed by 'Calva' and 'das Viñas'. 'Boullona' and 'Touro' form a small conglomerate, attached to the three above. Moreover, there are two cultivars which are less closely related 'Longal', and 'Monfortina', clearly differentiated from the clusters formed by the rest of the cultivars considered.

Conclusions

All the quantitative variables determined on leaves proved to be significant for differentiating cultivars. Of the biometrical parameters of flowers, only catkin length was useful, while in fruits, all except the volume of 100 fruits and density established significant differences among the 15 cultivars. We observed significant differences in weight, width, height and thickness of the fruit when comparing chestnuts of the same cultivar in different harvest years (1994 and 1997). Biometrical parameters of leaves and flowers accounted for 77.5% of total variance among these cultivars. Cluster analysis enabled us to group 13 of the 15 cultivars in to 4 different groups: the first cluster includes the cultivars 'Inxerta', 'Vilamaesa', 'Blanca' and 'Foleiro', with 'Soutiña' very near; a second group is formed by 'Corronchuda', 'Famosa' and 'das Viñas' and a fourth group with 'Boullona' and 'Touro'. 'Longal' and 'Monfortina' would form separate groups, since the euclidean distance that separates them from the above clusters is considerable.

Table 6. Comparison of some variables related to fruit morphology, between the harvest of 1994 and 1997, for the cultivars studied.

	Width	Height	Thickness	Unit weight
BERMELLA				
Means for the harvest of 1994	3.18	2.92	1.97	10.94
Means for the harvest of 1997	3.23	2.86	1.88	10.01
Variation with respect to the harvest of 1994 (%)	1.57	2.05	3.08	8.5
CALVA				
Means for the harvest of 1994	3.52	3.25	2.30	13.14
Means for the harvest of 1997	3.16	2.83	1.85	9.56
Variation with respect to the harvest of 1994 (%)	10.23	12.92	19.56	27.24
FAMOSA				
Means for the harvest of 1994	3.42	3.05	2.03	13.88
Means for the harvest of 1997	3.07	2.71	1.80	8.62
Variation with respect to the harvest of 1994 (%)	10.23	11.11	11.3	37.89
LONGAL				
Means for the harvest of 1994	2.91	3.49	1.84	11.08
Means for the harvest of 1997	2.64	2.92	1.59	6.58
Variation with respect to the harvest of 1994 (%)	9.28	16.33	13.59	40.62
SOUTIÑA				
Means for the harvest of 1994	3.25	3.40	2.11	13.44
Means for the harvest of 1997	3.03	2.77	1.85	8.04
Variation with respect to the harvest of 1994 (%)	6.77	18.53	12.34	40.18
TOURO				
Means for the harvest of 1994	3.09	3.28	1.90	10.90
Means for the harvest of 1997	3.22	2.56	2.10	10.36
Variation with respect to the harvest of 1994 (%)	4.21	21.9	10.05	4.95
VILAMAESA				
Means for the harvest of 1994	3.48	3.28	2.05	15.41
Means for the harvest of 1997	2.99	2.69	1.62	7.12
Variation with respect to the harvest of 1994 (%)	14.1	17.99	20.97	53.80

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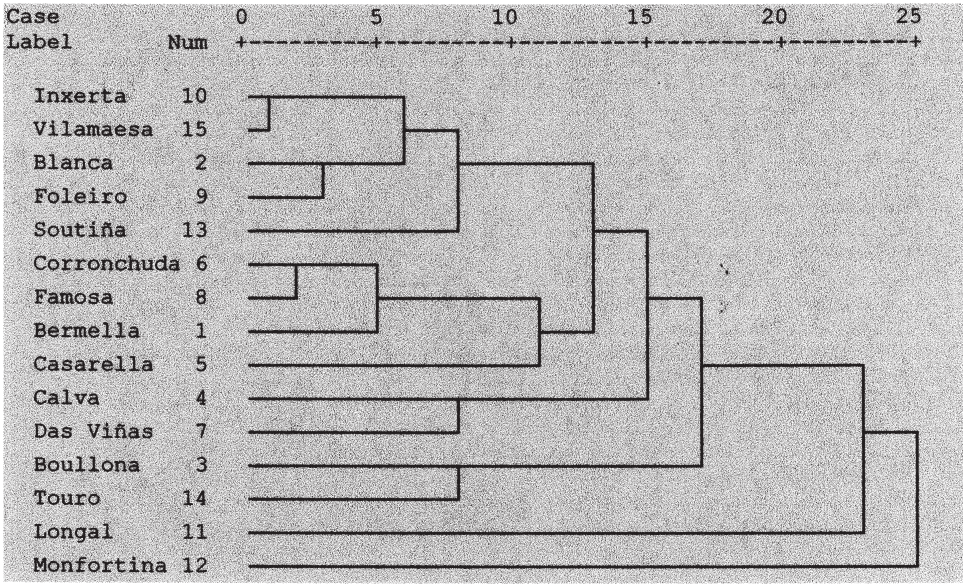
Figure 1. Dendrogram using Average Linkage (Between Groups. Rescaled Distance Cluster Combine.



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