Morphological and phenological description of 38 sweet chestnut cultivars (*Castanea sativa* Miller) in a contemporary collection

P. Furones-Pérez and J. Fernández-López*

Centro de Investigación Forestal de Lourizán. Consellería de Medio Rural. Xunta de Galicia.
Apdo. de Correos 127. 36080 Pontevedra. Spain

Abstract

Thirty eight traditional chestnut cultivars, from a contemporary collection, were described using nine characteristics, seven of which are included in the guidelines for carrying out tests of distinctness, homogeneity and stability of chestnut established by the International Union for the Protection of New Varieties of Plants (UPOV). The nine variables were chosen from among 13 characteristics evaluated in the collection with regard to the criteria for distinctness, uniformity and stability. The evaluations were carried out over the years 2003, 2004 and 2005, in two plantations, situated in the northwest of the Iberian Peninsula. Each mean value obtained for each cultivar, plantation and year were assigned a state and numerical number using the UPOV system or proposed new descriptors. No cultivar showed a very late time of leaf bud burst nor a very late time for the start of male and female flowering, nor a strong penetration of the seed coat into embryo. In five variables there was no or few differences among years and between plantations. Consequently they can be evaluated at one site in one year. These characters were: filament length of male flowers, percent of chestnuts with a split pericarp, the degree of penetration of the seed coat into the embryo, fruit shape and the ratio of hilum length to hilum width. Of the remaining four variables, three were phenological (time of leaf bud burst, time of beginning of male and female flowering) and one related to fruit size (size of fruit hilum). They varied among years and between plantations and consequently need to be evaluated under contrasting site conditions for a minimum number of years.

Additional key words: cultivated varieties, descriptor, genetic resources, UPOV.

Resumen

Descripción morfológica y fenológica de una colección de 38 cultivares de castaño (*Castanea sativa* Miller)

Se describen 38 cultivares tradicionales de castaño de una colección coetánea utilizando nueve características, siete de ellas incluidas en la guía de la *International Union for the Protection of New Varieties of Plants* (UPOV). Las evaluaciones se realizaron durante tres años sucesivos, 2003, 2004 y 2005, en dos plantaciones, Agrovello y Sergude, situadas en el Noroeste de la Península Ibérica. A cada uno de los valores medios obtenidos para cada cultivar, sitio y año se les asignó un estadio y una nota numérica utilizando las escalas UPOV o bien las propuestas por nosotros para nuevas características. Ningún cultivar presenta brotación ni floración masculina y femenina muy tardía, así como tampoco penetración del tegumento en el embrión de tipo fuerte. Cinco variables presentaron poca o ninguna diferencia entre años ni entre plantaciones y por lo tanto, su evaluación se puede hacer en un solo sitio, sólo un año. Estas variables fueron la longitud del filamento de la flor masculina, el porcentaje de castañas con el pericarpio reventado, el grado de penetración del tegumento en el embrión, la forma de la castaña y la relación entre la longitud y el ancho del hilum. Las restantes cuatro variables, tres de fenología (brotación, floración masculina y floración femenina) y una relacionada con el tamaño del fruto (tamaño del hilum) mostraron variaciones entre años y entre plantaciones y por lo tanto, deben ser evaluadas contrastando diferentes condiciones de sitio mediante un número mínimo de años.

Palabras clave adicionales: descriptor, recursos genéticos, UPOV, variedades cultivadas.

Abbreviations used: DUS (distinctness, uniformity and stability), PCA (principal components analysis), UPOV (International Union for the Protection of New Varieties of Plants).

Introduction

With regard to plant genetic resources, a descriptor is defined as an attribute, characteristic or measurable feature used in a germplasm bank accession (Biodiversity International, 2007). The use of lists of well defined and rigorously tested descriptors greatly simplifies operations associated with data registration with regard to the characterization and evaluation of cultivars. Elaboration of lists of descriptors is a dynamic, open process and standardization of descriptors is essential so that the characterization is of universal value.

Each descriptor consists of a name, a state and a method explaining how the descriptor should be measured and registered (Biodiversity International, 2007). Descriptor states are different values that can be assigned and may correspond to a quality, a measurable attribute or a code. To facilitate documentation descriptor states are assigned a numerical code, the note. The descriptor method describes in detail how and under what conditions the descriptor should be measured and noted, and includes the objective, conditions and sampling method. Description of the method facilitates the correct interpretation of the results and provides a universal and consistent protocol. It is essential to evaluate the character in a number of randomly selected plants or in a representative sample of as wide a range of variation as possible. The International Union for the Protection of New Varieties of Plants (UPOV) technical guidelines have been developed in order to elaborate standardized descriptions for the evaluation of distinctness, uniformity and stability (DUS) of plant material (Biodiversity International, 2007). The International Convention for the Protection of Plant Varieties (UPOV, 1991) defines distinctness, uniformity and stability in Articles 7, 8 and 9 respectively. The UPOV guidelines for chestnut (UPOV, 1989) includes 39 descriptors. Varieties included in lists of registered commercial varieties must be described with UPOV descriptors. The only official European list of commercial chestnut varieties is French (Chapa, 1987).

Many of the agronomic descriptions of chestnut cultivars, in the literature, were made *in situ*, on trees of different ages, situated in different environments (Bassi and Pellegrino, 1991; Pereira-Lorenzo *et al.*, 1996a, 1996b, 2001, 2006; Pereira-Lorenzo and Fernández-López, 1997a; Díaz Hernández, 2002; Pereira-Lorenzo and Ramos-Cabrer, 2003; Ramos-Cabrer *et al.,* 2003; Queijeiro *et al.,* 2006; Ertan, 2007;

Martín *et al.,* 2007). However, most of the characters of interest are quantitative characters that are greatly affected by environmental factors and therefore *in situ* characterizations are used as a prior step in characterization of a collection, in which decisions are made as regards which ortets to include in the collections. The only published characterization of a chestnut collection was carried out in Portugal (Valdivieso, 2000).

The objective of this study was description of a contemporary collection of 38 traditional chestnut cultivars on the basis of nine characteristics studied over 3 years and previously tested for use as descriptors in the UPOV, DUS test. Characteristics that did not show enough capacity for distinctness or had too low uniformity or stability were not used for description (Furones-Pérez and Fernández-López, 2009). The objective was therefore to describe the 38 chestnut cultivars for nine characteristics that differed significantly among the cultivars, seven of which are in the UPOV guidelines and two were proposed new characteristics. A state was assigned for each scale and characteristic.

Material and methods

Collections

The study was carried out with a collection of 38 Galician traditional chestnut-producing cultivars (*Castanea sativa* Mill.) grafted onto a hybrid clone resistant to ink disease, CHR-151 (*Castanea crenata* Siebold & Zucc. × *Castanea sativa* Mill.) (Pereira-Lorenzo and Fernández-López, 1997b; Miranda-Fontaíña *et al.*, 2007). The plantation was established in 1997 at two sites in northwest Spain, with spacing of 9×9 m, in a two-block randomized design, with at least one tree per cultivar and block. Not all of the cultivars in the plantations were used in the analysis. The following criteria were used to select cultivars for analysis: i) at least two trees of each cultivar in each plantation; ii) all trees, of each cultivar, were identical for all loci (by 10 isoenzyme systems) and catkin type.

The number of cultivars selected at the Agrovello and Sergude plantations were 12 and 35, respectively. Nine of these were common to both plantations. The Agrovello plantation is located in Lourizán (Pontevedra) and the Sergude plantation in Boqueixón (A Coruña).

The cultivar denominations, number of trees per plantation, name and geographic coordinates and altitude at the origin of each cultivar used in the analysis of each plantation are shown in Table 1. Both plantations are situated in the European Atlantic climatic subregion VI(V) (Allue, 1990). They are in the temperate hyperoceanic bioclimatic zone, according to the classification of bioclimatic zones of Europe (Rivas-Martínez *et al.*, 2004). Climate in the zone is characterized by a strong oceanic influence and is temperate and mild, with high levels of rainfall (Table 2). Mean annual rainfall in Agrovello is 1,449 mm, with a mean annual temperature of 14.8°C and a mean temperature fluctuation of 10.5°C. Mean annual rainfall in Sergude is 1,231 mm, with a mean annual temperature of 13.5°C and a mean temperature fluctuation of 10.9°C (Fig. 1).

Table 1. Denominations, number of trees per plantation (Agrovello or Sergude), name, geographic coordinates and altitude of the location of origin for each cultivar

Cultivar	Number of trees		Range of latitudes [N] (°)	Range of longitudes [W] (°)	Range of altitudes (m)	Localities
	Agrovello	Sergude				
Amarelante 1	7	3	42.32-41.97	7.62-7.02	950-550	Manzaneda, Mezquita
Amarelante 2		2	42.25	7.15	840	Bolo
Amarelante 3		2	42.17	7.07	920	Viana
Amarelante 4		4	42.88-42.87	7.05-7.02	570	Cervantes
Anaxa		2	42.12	7.13	860	Viana
Bermella		2	42.02	7.30	610	Riós
Blanca 1	3	4	42.50-42.42	7.38-7.05	700-450	Quiroga, Rivas, Vilamartín
Blanca 3		2	42.35	7.16	542	Larouco
Calva 3		3	42.25	7.15	840	Bolo
Courelá		2	42.38	7.32	820	Río, Folgoso
De Lemos		2	42.52	7.73	450	Parada, Folgoso, Carballedo
Unknown		2	—	—	—	Bolo
Famosa 1		2	41.92	7.23	860	Riós, Vilardevós
Famosa 2	2		—	—	—	Gudiña
Garrida 2		2	42.45	7.38	620	Ribas
Garrida 3		2	42.62	7.87	610	Chantada, Carballedo
Inxerta	4	8	42.32-42.17	7.22-7.07	950-550	Bolo, Manzaneda, Viana
Longal	4	2	42.02-41.90	7.22-7.18	870-810	Gudiña, Riós, Vilardevós
Loura	2	2	43.17-42.62	7.87-7.08	610-570	Chantada, Fonsagrada
Luguesa	4		42.75-42.58	7.32-7.23	770-600	Baralla, Folgoso, Samos
Negral 1	6		42.33	6.85	550	Barco, Carballeda, Rubiá
Negral 3		2	42.35	7.16	542	Larouco
Parede 1	5	4	43.08-42.17	7.18-6.87	920-400	Baralla, Becerreá, Cervantes, Fonsagrada, Navia
Parede 2		2	42.97	7.00	440	Navia
Praga D'afora		4	42.33	7.17-7.10	820-550	Bolo
Praga do Bolo		2	42.35	7.08	470	Bolo
Puga		2	42.30	7.23	670	Manzaneda
Raigona	3	3	42.38-42.03	7.20-6.88	780-600	Carballeda, Folgoso, Rubiá
Rapada 1	5	6	42.38-42.03	7.47-6.97	860-570	Bolo, Teixeira, Viana
Rapada 3		2	42.47	7.10	820	Vilamartín
Rozada		2	42.03	6.97	750	Bolo
Salnesa		2	42.38	7.10	480	Petín
Serodia		4	42.93-42.92	7.28-7.27	650	Baralla
Ventura	3	2	42.02-41.97	7.18-7.02	985-810	Gudiña, Mezquita
Verde 1		3	—	—	—	Folgoso, Samos
Verde 2		2	43.05	7.00	450	Navia
Verde 3		2	42.33	7.17	550	Bolo
Xábrega		3	42.33	7.37	1,000	Chandrexa

Table 2. Average rainfall and temperatures during the study years at each plantation

Month	Agrovello		Sergude	
	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)
January	162.0	9.9	128.5	8.4
February	63.7	9.3	66.7	7.6
March	104.7	11.8	94.7	10.4
April	139.0	13.4	127.3	12.1
May	74.7	16.0	78.0	14.5
June	59.0	20.2	60.3	19.5
July	55.3	20.4	40.3	19.4
August	75.7	21.3	62.7	20.2
September	64.7	18.9	47.7	18.2
October	337.7	15.2	255.0	13.8
November	185.7	11.8	150.0	10.2
December	127.0	9.5	119.3	8.2

Observations

Phenological observations of each tree were recorded, once or twice a week. The number of days on which observations on flushing were made was 18 in 2003, 17 in 2004 and 15 in 2005. Observations on flowering were made 23 times in 2003, 24 in 2004 and 12 in 2005. During fruiting, the dates that chestnuts were collected from each tree were recorded.

Filament length (FilamentL) was recorded for each tree in 2003, 2004 and 2005, following the classification of Solignat and Chapa (1975). This was made on the basis of the length of the stamen filaments in male flowers of male catkins.

Nut characteristics were recorded in 2003 and 2004. The number and weight of healthy chestnuts in the sample and the number of healthy chestnuts with a visibly split pericarp were recorded for whole tree production. A weighted mean value for the quantity of chestnuts collected was calculated for each tree to obtain the following variables: the number of chestnuts per kg (NutSize) and the proportion of split chestnuts (NutSplit). Fifteen lateral chestnuts (UPOV, 1989) were taken from each healthy nut sample and the following measurements were made: number of embryos, to determine embryony (Embryony); the degree of penetration of the seed coat into the embryo (TegumentP), on a numerical scale, 0: no penetration, 1: weak penetration visible ≤ 2 mm, 2: strong penetration visible > 2 mm (Fig. 2a); chestnut width, chestnut length (NutLength) and hilum width and hilum length. The following variables were calculated from the latter: the ratio of chestnut length:chestnut width (NutShape, Fig. 2b), hilum width × hilum length (HilumSize, Fig. 2c) and ratio hilum length:hilum width (HilumLW, Fig. 2d).

Descriptors

Prior to description of the cultivar collection 13 morphological and phenological traits were evaluated in accordance with the UPOV descriptors and others previously recommended on the basis of their potential usefulness in the DUS test. The genetic and environmental components of the variability and the genotype ×
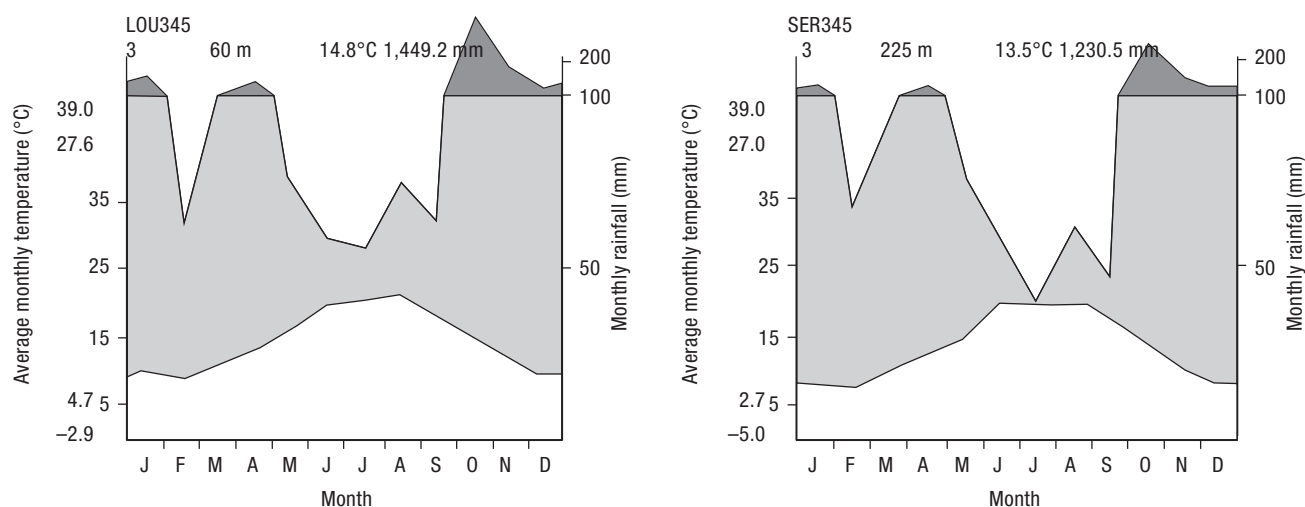

Figure 1. Walter-Lieth climatic diagrams for Agrovello (LOU345) and Sergude (SER345) plantations in the years 2003, 2004 and 2005 (program PROCLI v1.0, http://www.uhu.es/03009/procli/procli.html).
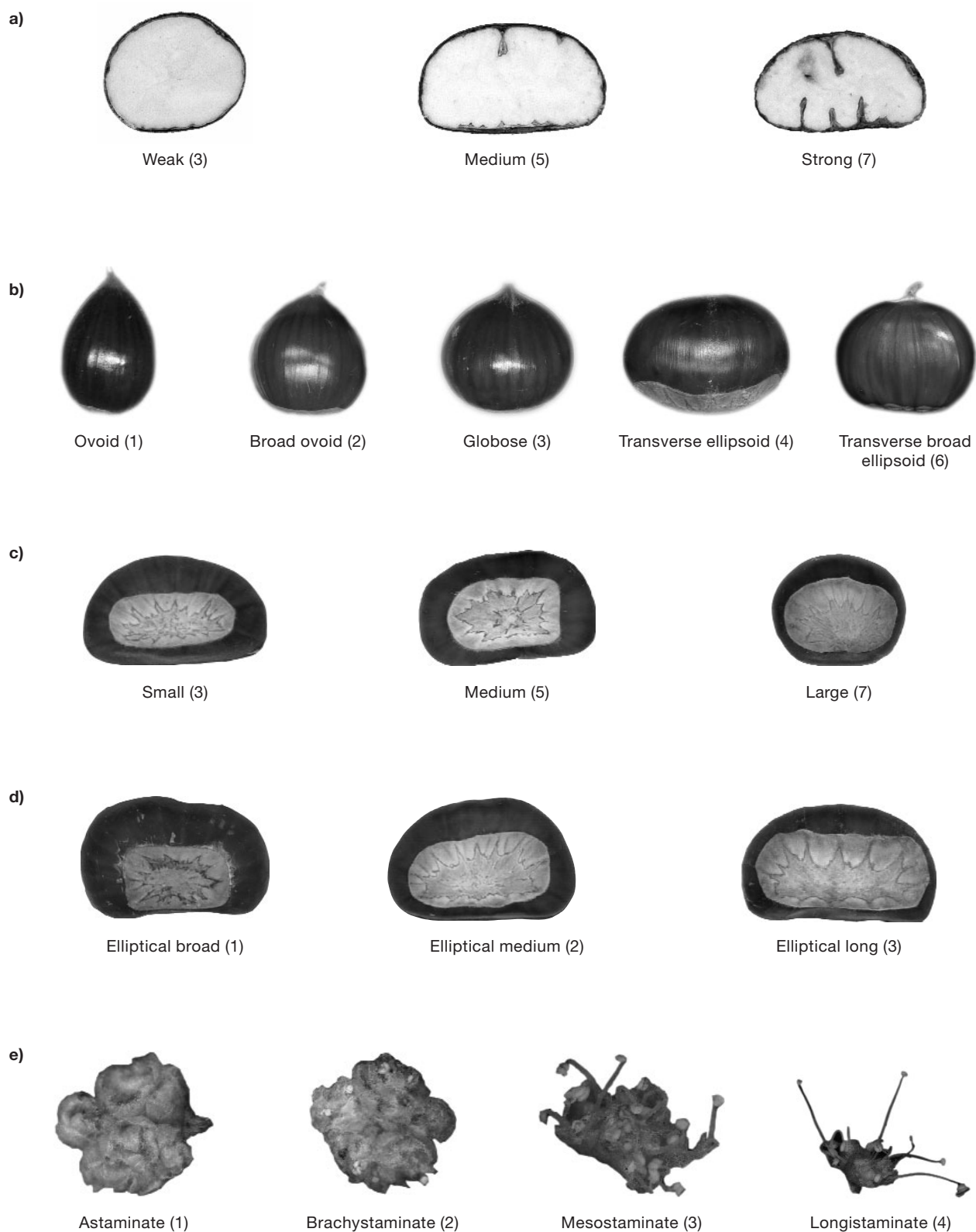
a)

Weak (3)   Medium (5)   Strong (7)

b)

Ovoid (1)   Broad ovoid (2)   Globose (3)   Transverse ellipsoid (4)   Transverse broad ellipsoid (6)

c)

Small (3)   Medium (5)   Large (7)

d)

Elliptical broad (1)   Elliptical medium (2)   Elliptical long (3)

e)

Astaminate (1)   Brachystaminate (2)   Mesostaminate (3)   Longistaminate (4)

Figure 2. The states (UPOV notes) of the characteristics a) degree of seed coat penetration into the embryo (characteristic 30), b) fruit shape (characteristic 31), c) hilum size (characteristic 32), d) HilumLW (characteristic 40) and e) filament lenght one male flower glomerule in male catkins (characteristic 9 modified).

environment interaction were estimated by ANOVA, to determine the usefulness of the properties of different descriptors for the DUS test (Furones-Pérez and Fernández-López, 2009). With the aim of classifying the variables according to their value in the DUS test, the heritability values for cultivars H^2c, the plasticity coefficient pl, values of the plasticity heritability H^2pl_c and the error variances were classified into five groups (Furones-Pérez and Fernández-López, 2009). FruitRipening, NutSize, NutLength and Embryony did not show a good capacity for distinguishing among cultivars in the collection (Furones-Pérez and Fernández-López, 2009).

The chestnut cultivars were described using nine characteristics, seven of which are in the UPOV guidelines. For each plantation mean values for each characteristic were calculated for each cultivar and year or for the three year period of the study, depending on whether or not year was significant in the analysis of variance (Furones-Pérez and Fernández-López, 2009).

The seven characteristics in the UPOV guidelines are time of leaf bud burst (BudBurst, UPOV 8), filament length of the male flower (FilamentL, UPOV 9), time of beginning of male flowering (MFlowering, UPOV 11), time of beginning of female flowering (FFlowering, UPOV 12), degree of penetration of seed coat into embryo (TegumentP, UPOV 30), fruit shape (NutShape, UPOV 31) and size of the fruit hilum (HilumSize, UPOV 32). The two new characteristics proposed are the ratio of hilum length:hilum width (HilumLW) and the percentage of chestnuts with a split pericarp (NutSplit). For each characteristic a scale of states was established and assigned the corresponding numerical scores (notes).

The scale for BudBurst (UPOV 8) includes five states based on the suggestions of Bergonoux *et al.* (1978). The scale used for FilamentL (UPOV 9 modified, Fig. 2e) includes four states, following the classification of Solignat and Chapa (1975) and was on the basis of the filament length of the stamens in male flowers of male catkins. Astaminate catkins, not considered in the UPOV scale, were assigned a value of 1. The scales for MFlowering (UPOV 11) and FFlowering (UPOV 12) include five states based on the suggestions of Solignat and Chapa (1975). For NutSplit a 3 state scale was established. As with HilumSize (UPOV 32, Fig. 2c) the range of values for matching the value calculated for NutSplit and the scores assigned were calculated by dividing the difference

between the maximum and minimum values by 3. This classification was valid for comparison among cultivars in the collection studied. The scale for TegumentP (UPOV 30, Fig. 2a) comprises three states. The scale for NutShape (UPOV 31, Fig. 2b) includes five states. Chestnut shape was calculated as a function of the ratio between chestnut length and width, expressed as a percentage. The scale for HilumSize (UPOV 32) comprised three states. As the UPOV guidelines (UPOV, 1989) for the DUS test for chestnut do not specify how this classification should be carried out, the values for matching the calculated value of the ratio of hilum length and width and the scores were obtained by dividing the difference between the maximum and minimum values by 3. This classification is valid for comparison among the cultivars in the study collection. For HilumLW (Fig. 2d) a 3 point scale of states was established for the ratio between hilum length and width. Scores were obtained by dividing the difference between maximum and minimum values by 3. This classification was valid for comparisons among the cultivars studied in the collection. The description, scale of states and notes of the nine descriptors are shown in Table 3.

Cultivar classification

The usefulness of the set of variables for differentiating cultivars from each plantation was studied using principal components analysis (PCA). For purposes of classification it is necessary to demonstrate if the cultivars are different or similar for at least 1 of the chosen descriptors and the variables used are the notes.

A correlation matrix was calculated from the notes for each descriptor per cultivar and for each plantation. The notes were assigned according to averages for each year or for all three years of study if the year factor was significant or not in the previous ANOVA analyses (Furones-Pérez and Fernández-López, 2009). The PCA was carried out using the PRINCOMP procedure of SAS (2006). The distance matrix for both plantations was calculated from the selected principal components. The Mahalanobis distance was used as a measure of dissimilarity among cultivars. From this matrix, a cluster analysis was performed using the UPGMA clustering method in the SAS (2006) CLUSTER procedure. The phenetic dendrograms showing the relationships, among cultivars, were obtained by the SAS (2006) TREE procedure.

Table 3. Description and scale of states of seven UPOV descriptors and two new ones

UPOV number	Descriptor name 1	Descriptor name 2	Descriptor state	Scale	Note
8	Time of leaf bud burst	BudBurst	Very early	Before or on 25 March	1
			Early	Between 26 March and 15 April	3
			Medium	Between 16 April and 30 April	5
			Late	Between 1 May and 15 May	7
			Very late	After 15 May	9
9 Modified	Stamen filament length of male flower	FilamentL	No filament	Astaminate	1
			Short (1-3 mm)	Brachystaminate	2
			Medium (3-5 mm)	Mesostaminate	3
			Long (5-7 mm)	Longistaminate	4
11	Time of beginning of male flowering	MFlowering	Very early	Before or on 15 June	1
			Early	Between 16 and 30 June	3
			Medium	Between 1 and 15 July	5
			Late	Between 16 and 31 July	7
			Very late	Later than 31 July	9
12	Time of beginning of female flowering	FFlowering	Very early	Before or on 15 June	1
			Early	Between 16 and 30 June	3
			Medium	Between 1 and 15 July	5
			Late	Between 16 and 31 July	7
			Very late	Later than 31 July	9
30	Degree of penetration of seed coat into the embryo	TegumentP	Weak	See Fig. 2	3
			Medium		5
			Strong		7
31	Fruit: shape	NutShape	Ovoid	< 100	1
			Broad ovoid	> 100 and < 110	2
			Globose	$= 100$	3
			Transverse ellipsoid	> 120	4
			Transverse broad ellipsoid	≥ 110 and ≤ 120	5
32	Fruit: size of hilum	HilumSize	Small	≥ 131.67 and < 273.36	3
			Medium	≥ 273.36 and ≤ 415.05	5
			Large	> 415.05 and ≤ 556.74	7
Not included	Fruit: shape of hilum	HilumLW	Elliptical broad	≥ 1.7 and <1.9	1
			Elliptical medium	≥ 1.9 and ≤ 2.1	2
			Elliptical long	> 2.1 and ≤ 2.3	3
Not included	Percent of chestnuts with a split pericarp	NutSplit	Low	≥ 0.0 and <15.0	3
			Medium	≥ 15.0 and ≤ 29.9	5
			High	> 29.9 and ≤ 44.9	7

When using PCA for interpretation of multivariate data, it is necessary to select the components that have practical significance. A simple, although arbitrary, rule that has proved useful, in practice, is to only consider components that have eigenvalues of 1.0 or more as having practical significance (Jeffers, 1967). The eigenvectors values of these components are scaled by dividing each eigenvector by the maximum value so the maximum weighting is +1. Interpretation of the weightings may then be made fairly simply, by considering variables that have relatively high positive or negative weighting (> 0.7) as constituting an index of the combined action, or in contrast, of the original variables, provided this arbitrary criterion is not over-stressed (Jeffers, 1967).

Results

Cultivar descriptions

Attribution of states for the seven UPOV and two proposed new characteristics for cultivar descriptions in the Agrovello and Sergude plantations are given in Tables 4a and 4b.

At Agrovello plantation, *BudBurst* was stable across years, except in the cultivars Longal and Ventura. There was late BudBurst in two of the 12 stable cultivars at Agrovello plantation (Amarelante1 and Famosa2) and medium BudBurst in eight cultivars (Blanca1, Inxerta, Loura, Luguesa, Negral, Parede1, Raigona and Rapada1) (Table 4a and Fig. 3a). By contrast, at Sergude plantation, BudBurst was unstable across years for 31 of the 35 cultivars. The four stable cultivars were: De Lemos, with late BudBurst, and Parede2, Rozada and Verde1 with medium BudBurst (Table 4b and Fig. 3a).

FilamentL was stable across years and between plantations, except in the cultivars, Longal and Raigona, which varied from brachystaminate at Agrovello plantation to mesostaminate at Sergude plantation (Tables 4a and 4b). Among the 36 cultivars, 6 were classified as longistaminate, 21 as mesostaminate, 7 as brachystaminate and 2 as astaminate (Fig. 3b). Longistaminate cultivars were Blanca3, De Lemos, Negral1, Parede2, Salnesa and Serodia. Mesostaminate cultivars were Amarelante1, Amarelante2, Amarelante3, Anaxa, Bermella, Calva3, Courelá, Unknown, Garrida2, Inxerta, Loura, Luguesa, Negral3, Parede1, Puga, Rapada1, Rapada3, Rozada, Ventura, Verde2 and Verde3. Brachystaminate cultivars were Blanca1, Famosa1, Famosa2, Garrida3, Praga D'afora, Praga Do Bolo and Xábrega and astaminate cultivars were Amarelante4 and Verde1 (Tables 4a and 4b).

Male flowering at Agrovello was stable across years in 8 cultivars and unstable in 4 cultivars (Table 4a). Medium male flowering was observed in 6 of the stable cultivars (Amarelante1, Famosa2, Inxerta, Longal, Raigona and Ventura) and early male flowering in Blanca1 and Negral1 (Fig. 3c). At Sergude plantation, 27 of the 32 cultivars were stable across years and showed medium male flowering (Amarelante1, Amarelante2, Amarelante3, Anaxa, Bermella, Calva3, Courelá, De Lemos, Unknown, Garrida2, Inxerta, Longal, Loura, Negral3, Parede1, Praga D'afora, Praga Do Bolo, Raigona, Rapada1, Rapada3, Rozada, Salnesa, Serodia, Ventura, Verde2, Verde3 and Xábrega) (Table 4b). Cultivars that were unstable across years were Blanca3, Famosa1, Garrida3, Parede2 and Puga (Fig. 3c).

The descriptor *female flowering* at Agrovello was stable in 5 cultivars and unstable in 7. Medium female flowering was observed in 4 stable cultivars (Amarelante1, Famosa2, Inxerta and Negral1) and early female flowering in Ventura. The 7 unstable cultivars were Blanca1, Longal, Loura, Luguesa, Parede1, Raigona and Rapada1 (Table 4a). At Sergude and during the 3 years of the study, female flowering was stable in 31 cultivars and variable in 4. Stable cultivars were Amarelante1, Amarelante2, Amarelante3, Anaxa, Bermella, Blanca1, Blanca3, Calva3, Courelá, De Lemos, Unknown, Famosa1, Garrida2, Garrida3, Inxerta, Longal, Loura, Negral3, Parede1, Praga D'afora, Praga Do Bolo, Puga, Raigona, Rapada1, Rozada, Salnesa, Ventura, Verde1, Verde2, Verde3 and Xábrega. Cultivars that varied across years were Amarelante4, Parede2, Rapada3 and Serodia (Table 4b and Fig. 3d).

Percentage of chestnuts with split pericarp was stable between plantations and across years, with low values of between 0% and 14%. Differences between plantations were only observed for the cv. Blanca1, 45% at Agrovello (high) and 24% at Sergude (medium) (Table 4a, 4b and Fig. 3e).

Penetration of the seed coat into the embryo was stable and medium between plantations and across years, except for Famosa2 at Agrovello and Famosa1, Unknown and De Lemos at Sergude where it was weak. There was no variation in classification of the 9 common cultivars between plantations, which were medium (Table 4a, 4b and Fig. 3f).

Chestnut shape was stable across years at both plantations. At Agrovello chestnut shape was classified as broad ovoid in 5 cultivars (Amarelante1, Inxerta, Longal, Parede1 and Ventura), transverse ellipsoid in 3 cultivars (Blanca1, Loura and Luguesa) and transverse broad ellipsoid in 4 (Famosa2, Negral1, Raigona and Rapada1) (Table 4a and Fig. 3g). At Sergude chestnuts were ovoid in 3 cultivars (Bermella, Longal and Negral3), broad ovoid in 10 cultivars (Amarelante1, Anaxa, Inxerta, Parede1, Parede2, Praga do Bolo, Puga, Rozada, Ventura and Verde3), transverse ellipsoid in 3 cultivars (Blanca1, Loura and Serodia) and transverse broad ellipsoid in the remaining 18 cultivars (Amarelante2, Amarelante3, Amarelante4, Blanca3, Calva3, Courelá, De Lemos, Unknown, Famosa1, Garrida2, Garrida3, Praga D'afora, Raigona, Rapada1, Salnesa, Verde1, Verde2 and Xábrega) (Table 4b and Fig. 3g). Cultivars common to the two plantations were stable, except for Longal in which

Table 4. Attribution of a state for the seven UPOV and two recommended characteristics for description of chestnut cultivars at the Agrovello and Sergude plantations

Cultivar	BudBurst	FilamentL	MFlowering	FFlowering	NutSplit	TegumentP	NutShape	HilumSize	HilumLW
a) *Agrovello*									
Amarelante1ᶜ	Late	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Small-medium	Medium elliptic
Blanca1ᶜ	Medium	Brachystaminate	Early	Early-medium	High	Medium	Transverse ellipsoid	Medium	Medium elliptic
Famosa2	Late	Brachystaminate	Medium	Medium	Low	Weak	Transverse broad ellipsoid	Medium	Long elliptic
Inxertaᶜ	Medium	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Large	Medium elliptic
Longalᶜ	Medium-late	Brachystaminate	Medium	Early-medium	Low	Medium	Broad ovoid	Small-medium	Medium elliptic
Louraᶜ	Medium	Mesostaminate	Early-medium	Early-medium	Low	Medium	Transverse ellipsoid	Large	Broad elliptic
Luguesa	Medium	Mesostaminate	Early-medium	Early-medium	Low	Medium	Transverse ellipsoid	Large	Broad elliptic
Negral1	Medium	Longistaminate	Early	Medium	Low	Medium	Transverse broad ellipsoid	Large	Medium elliptic
Parede1ᶜ	Medium	Mesostaminate	Early-medium	Early-medium	Low	Medium	Broad ovoid	Medium	Broad elliptic
Raigonaᶜ	Medium	Brachystaminate	Medium	Early-medium	Low	Medium	Transverse broad ellipsoid	Small	Medium elliptic
Rapada1ᶜ	Medium	Mesostaminate	Early-medium	Early-medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic
Venturaᶜ	Medium-late	Mesostaminate	Medium	Early	Low	Medium	Broad ovoid	Medium	Medium elliptic
b) *Sergude*									
Amarelante1ᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Small	Medium elliptic
Amarelante2	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Small	Long elliptic
Amarelante3	Early-medium	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Broad elliptic
Amarelante4	Early-medium	Astaminate	—	Early-medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic
Anaxa	Early-medium	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Medium	Medium elliptic
Bermella	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Ovoid	Small	Medium elliptic
Blanca1ᶜ	Medium-late	Brachystaminate	Medium	Medium	Medium	Medium	Transverse ellipsoid	Small	Medium elliptic
Blanca3	Early-medium	Longistaminate	Early-medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic
Calva3	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium-large	Long elliptic
Courelá	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium-large	Medium elliptic
De Lemos	Late	Longistaminate	Medium	Medium	Low	Weak	Transverse broad ellipsoid	Small-large	Medium elliptic
Unknown	Medium-late	Mesostaminate	Medium	Medium	Low	Weak	Transverse broad ellipsoid	Small	Broad elliptic
Famosa1	Medium-late	Brachystaminate	Medium-late	Medium	Low	Weak	Transverse broad ellipsoid	Medium	Medium elliptic
Garrida2	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Small-large	Broad elliptic
Garrida3	Medium-late	Brachystaminate	Medium-late	Medium	Low	Medium	Transverse broad ellipsoid	Medium-large	Broad elliptic
Inxertaᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Medium-large	Medium elliptic
Longalᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Ovoid	Small	Medium elliptic
Louraᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse ellipsoid	Medium-large	Medium elliptic
Negral3	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Ovoid	Small	Medium elliptic
Parede1ᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Medium	Broad elliptic
Parede2	Medium	Longistaminate	Early-medium	Early-medium	Low	Medium	Broad ovoid	Small	Broad elliptic
Praga D'afora	Medium-late	Brachystaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic
Praga Do Bolo	Medium-late	Brachystaminate	Medium	Medium	Low	Medium	Broad ovoid	Small-medium	Medium elliptic
Puga	Medium-late	Mesostaminate	Medium-late	Medium	Low	Medium	Broad ovoid	Small	Medium elliptic
Raigonaᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Small	Medium elliptic
Rapada1ᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Small-medium	Medium elliptic
Rapada3	Medium-late	Mesostaminate	Medium	Early-medium	—	—	—	—	—
Rozada	Medium	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Small-medium	Long elliptic
Salnesa	Medium-late	Longistaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Small	Medium elliptic
Serodia	Early-medium	Longistaminate	Medium	Early-medium	Low	Medium	Transverse ellipsoid	Medium-large	Medium elliptic
Venturaᶜ	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Medium	Medium elliptic
Verde1	Medium	Astaminate	—	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic
Verde2	Medium-late	Mesostaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Broad elliptic
Verde3	Early-medium	Mesostaminate	Medium	Medium	Low	Medium	Broad ovoid	Small	Medium elliptic
Xábrega	Medium-late	Brachystaminate	Medium	Medium	Low	Medium	Transverse broad ellipsoid	Medium	Medium elliptic

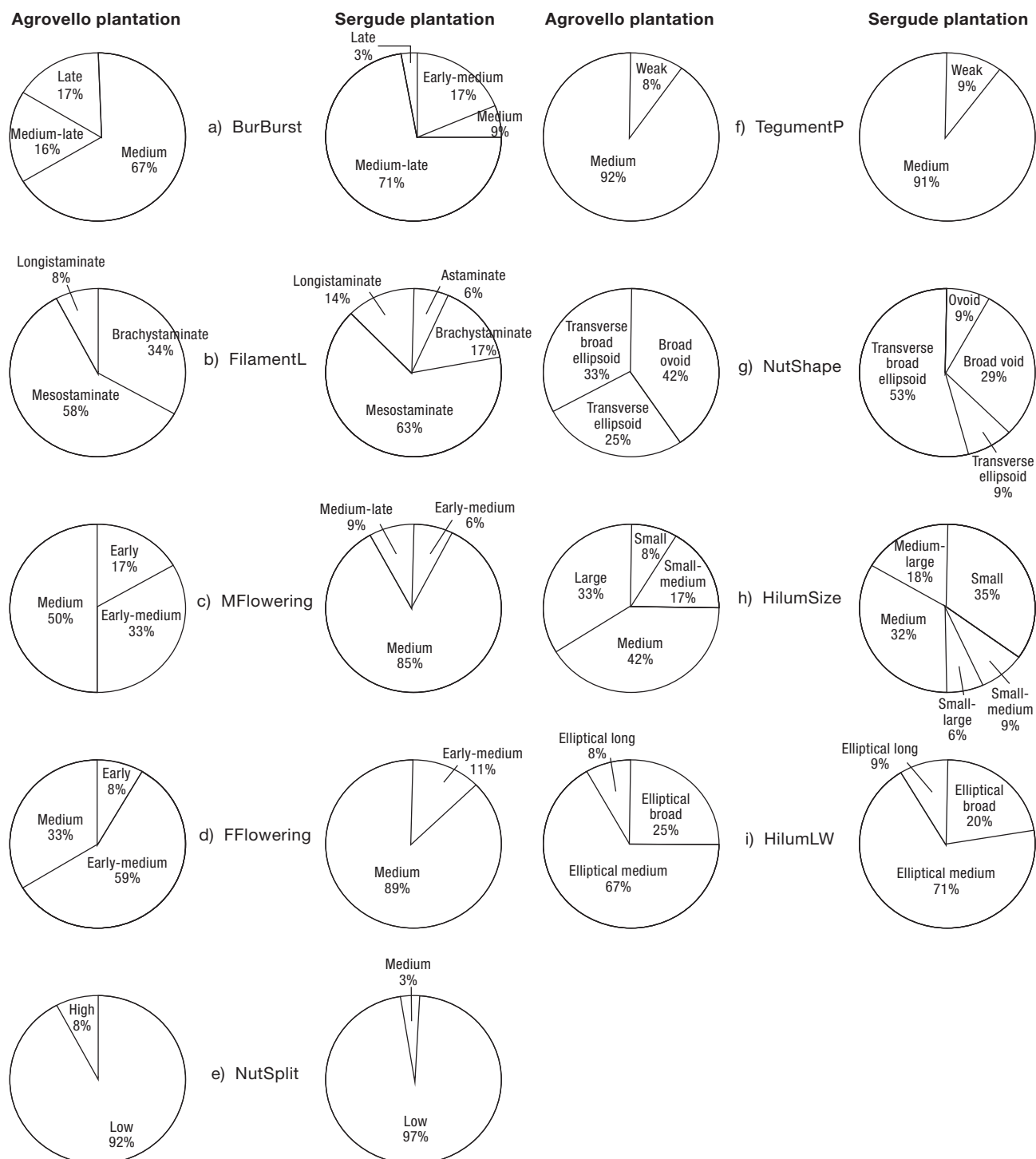ᶜ Cultivars common to both plantations.

Figure 3. Percentage of chestnut cultivars classified in different states for each characteristic considered of value to perform the DUS test. The number of cultivars was 38 and results are presented individually for each plantation. The states (UPOV notes) of the characteristics are shown for (top to bottom): a) time of leaf bud burst (BudBurst, characteristic 8), b) male flower: filament length (FilamentL, characteristic 9), c) time of beginning of male flowering (MFlowering, characteristic 11), d) time of beginning of female flowering (FFlowering, characteristic 12), e) percentage chestnuts with a split pericarp (NutSplit, proposed characteristic 41), f) fruit: degree of penetration of the seed coat into the embryo (TegumentP, characteristic 30), g) fruit: shape (NutShape, characteristic 31), h) fruit: size of hilum (HilumSize, characteristic 32) and i) ratio hilum length:hilum width (HilumLW, proposed characteristic 40).

chestnut shape varied from broad ovoid at Agrovello to ovoid at Sergude (Tables 4a, 4b). The 8 stable cultivars were Amarelante1, Inxerta, Parede1 and Ventura (broad ovoid), Blanca1 and Loura (transverse ellipsoid) and Raigona and Ventura (transverse broad ellipsoid).

At Agrovello, *hilum size* was stable across years in 10 cultivars and unstable in 2 (Amarelante1 and Longal). Hilum size was small in 1 stable cultivar (Raigona), medium in 5 cultivars (Blanca1, Famosa2, Parede1, Rapada1 and Ventura) and large in 4 (Inxerta, Loura, Luguesa and Negral1) (Table 4a and Fig. 3h). At Sergude, hilum size was stable across years in 23 cultivars and unstable in 11 (Calva3, Courelá, De Lemos, Garrida2, Garrida3, Inxerta, Loura, Praga do Bolo, Rapada1, Rozada and Serodia). In the stable cultivars hilum size was classified as small in 12 cultivars (Amarelante1, Amarelante2, Bermella, Blanca1, Unknown, Longal, Negral3, Parede2, Puga, Raigona, Salnesa and Verde3) and medium in 11 cultivars (Amarelante3, Amarelante4, Anaxa, Blanca3, Famosa1, Parede1, Praga D'afora, Ventura, Verde1, Verde2 and Xábrega) (Table 4b and Fig. 3h).

The descriptor *hilum shape* was stable across years. At Agrovello hilum shape was broad elliptic in 3 cultivars (Loura, Luguesa and Parede1) and medium elliptic in 9 (Amarelante1, Blanca1, Famosa2, Inxerta, Longal, Negral1, Rapada1, Raigona and Ventura) (Table 4a and Fig. 3i). At Sergude, the hilum was broad elliptic in 7 cultivars (Amarelante3, Unknown, Garrida2, Garrida3, Parede1, Parede2 and Verde2), medium elliptic in 24 cultivars (Amarelante1, Amarelante4, Anaxa, Blanca1, Bermella, Blanca3, Courelá, De Lemos, Famosa1, Inxerta, Longal, Loura, Negral3, Praga D'afora, Praga do Bolo, Puga, Raigona, Rapada1, Salnesa, Serodia, Ventura, Verde1, Verde2 and Xábrega,) and long elliptic in 3 (Amarelante2, Calva3 and Rozada) (Table 4b and Fig. 3i). Hilum shape was stable in 9 common cultivars in the plantations, except for Loura, which varied between broad elliptic at Agrovello to medium elliptic at Sergude. The hilum shape in cv. Parede1 was broad elliptic and in the other 7 cultivars (Amarelante1, Blanca1, Inxerta, Longal, Raigona, Rapada1 and Ventura) it was medium elliptic (Tables 4a, 4b).

Cultivar classification

In the first study with nine variables, the first four eigenvalues were > 1, and explained more than 68% of the total variation (Table 5). In the second study with

Table 5. Eigenvectors[1] for first four components from nine variables of 35 chestnut cultivars at both plantations

Variables	Components (% variance)			
	1 (26.1%)	2 (16.8%)	3 (14.1%)	4 (11.3%)
BudBurst	0.997	0.105	0.147	–0.113
FilamentL	–0.792	0.104	–0.590	0.429
MFlowering	1.000	–0.254	–0.259	–0.470
FFlowering	0.858	0.083	–0.259	0.424
NutSplit	–0.142	–0.100	1.000	0.183
TegumentP	–0.781	–0.505	0.044	0.004
NutShape	0.213	1.000	0.115	0.152
HilumSize	–0.403	0.843	0.027	–0.102
HilumLW	0.515	–0.233	0.060	1.000

[1] The values shown are the result of dividing each vector by the maximum coefficient.

five variables, the first three eigenvalues were > 1, and explained about 71% of the total variation (Table 6).

The eigenvector values of these first components are given in Table 5 for the nine variables and in Table 5b for the five variables. In both studies, the first component is a measure of the filament length of the male flower and the degree of penetration of the seed coat into the embryo. Cultivars with high pollen production had a strong degree of penetration of seed coat into the embryo. Other characteristics affect the first component but were not common in both studies.

Thirty-two groups were defined with nine variables (Fig. 4). The following cultivars did not display any differences from each other: Garrida2 and Verde2, Praga D'afora and Xábrega, and Bermella and Negral3.

Twenty-three groups were defined with five variables (Fig. 5). The following cultivar groups did not display any differences from each other: Garrida2, Verde2 and Amarelante3; Praga D'afora and Xábrega; Bermella and Negral3; Courelá and Rapada1; Amarelante4 and

Table 6. Eigenvectors[1] for first three components from five variables of 37 chestnut cultivars at both plantations

Variables	Components (% variance)		
	1 (27.3%)	2 (23.7%)	3 (20.0%)
FilamentL	–0.786	–0.755	0.090
NutSplit	0.370	1.000	–0.398
TegumentP	–0.899	0.715	–0.193
NutShape	1.000	–0.487	–0.329
HilumLW	0.373	0.444	1.000

[1] The values shown are the result of dividing each vector by the maximum coefficient.
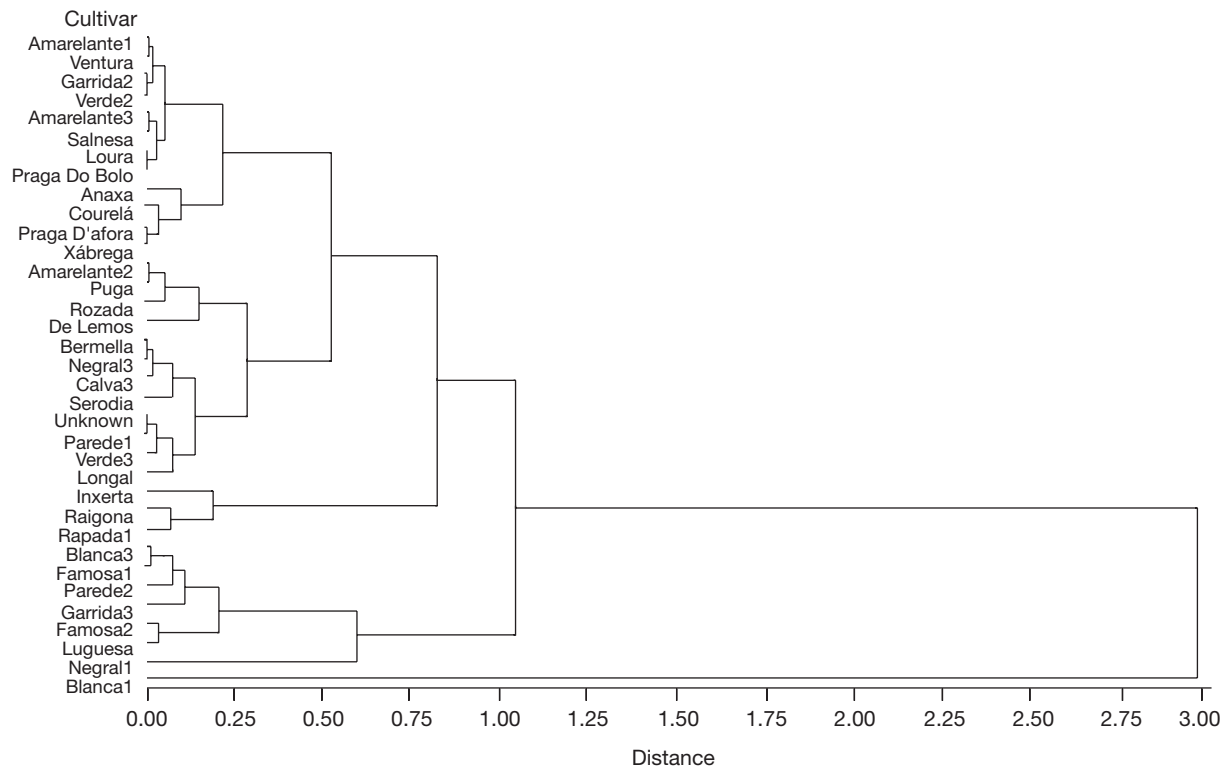
Figure 4. Average linkage dendrogram of 134 trees corresponding to 35 chestnut cultivars from both plantations based on Mahalanobis generalized distance for nine morphological and phenological variables.
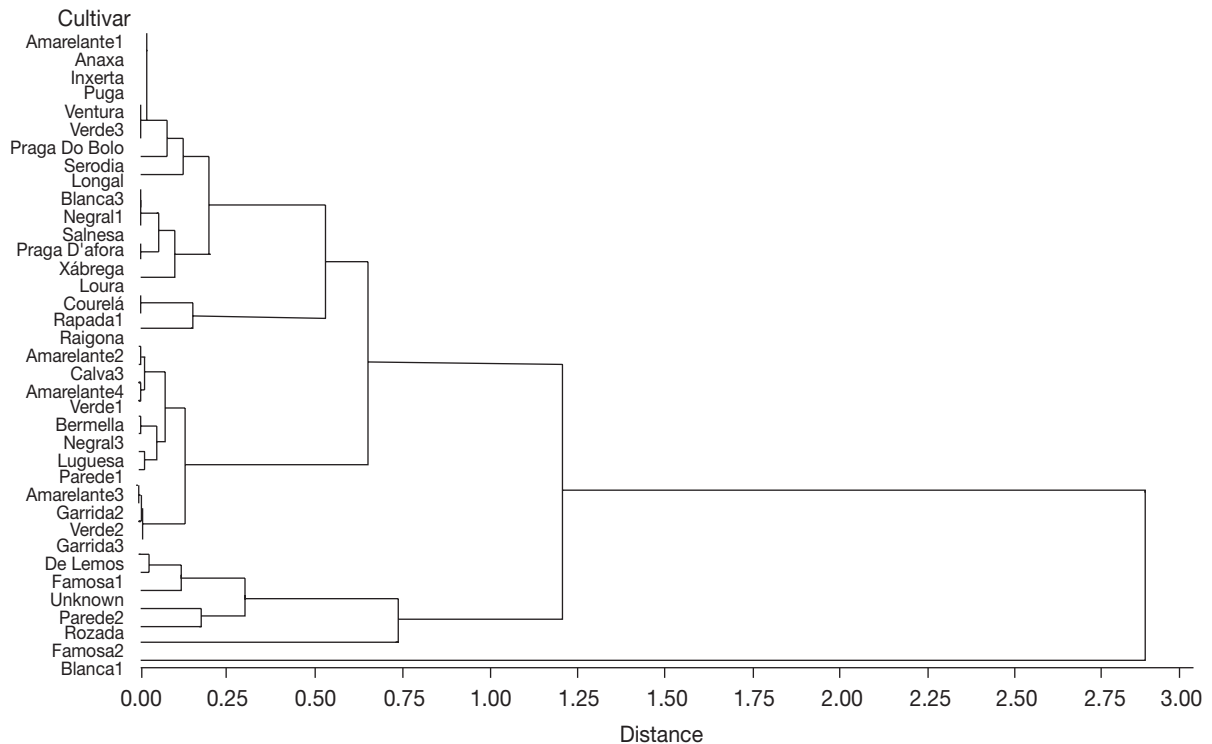

Figure 5. Average linkage dendrogram of 141 trees corresponding to 37 chestnut cultivars from both plantations based on Mahalanobis generalized distance for five morphological and phenological variables.

Verde1; Amarelante2 and Calva3; Blanca3, Negral1 and Salnesa; Amarelante1, Anaxa, Inxerta, Puga, Ventura and Verde3.

Discussion

Three of the six characteristics recommended by UPOV for use in the DUS test with chestnut varieties and their grouping were described in this study. The time of beginning of male flowering (MFlowering, UPOV 11), time of beginning of female flowering (FFlowering, UPOV 12) and fruit shape (NutShape, UPOV 31) showed a degree of variability that enabled cultivar classification. A further four characteristics, two in the UPOV technical guidelines for chestnut, *i.e.* degree of penetration of seed coat into embryo (TegumentP, UPOV 30) and size of fruit hilum (HilumSize, UPOV 32), and another two not included in the UPOV guidelines, percentage of chestnuts with a split pericarp (NutSplit) and the ratio hilum length:hilum width (HilumLW) were also useful for cultivar differentiation. Two additional characteristics, also recommended by UPOV, for grouping varieties, fruit embriony (Embriony, UPOV 27) and fruit size (NutSize, UPOV 36) did not vary in the collection study, as all varieties were monoembryonic and size was not uniform.

A group of variables did not vary among years at the same site and matched at both plantations. The variables include one male flower morphology variable —FilamentL— and four fruit variables —NutSplit, TegumentP, NutShape and HilumLW—. There was variation between the plantations for cultivars common to both. The differences for 4 of these variables were small and none were observed for TegumentP. This indicates that small differences lead to classification of common cultivars with different notes. These variables could be averaged and analyzed jointly by multivariate analysis.

All these characteristics have very good properties for the DUS test. FilamentL shows very high heritability, uniformity and stability. NutSplit, NutShape, TegumentP and HilumLW display high stability and heritability. However, assessment of the latter characteristics is more time-consuming and, because of the low degree of uniformity, a sample of adequate size for each tree must be evaluated (Furones-Pérez and Fernández-López, 2009).

The remaining four variables, three phenological variables (BudBurst, MFlowering and FFlowering) and

one fruit size variable (HilumSize) showed variations at each plantation for the different years of the study. These variables are the best descriptors for characterization in the collection because of their high heritability and moderate uniformity. The stability of these traits is low or moderate but is of a purely environmental origin. Accordingly, phenological characteristics were unstable (Furones-Pérez and Fernández-López, 2009), and are greatly affected by environmental factors and should only be evaluated in contemporary collections. They are good descriptors for application in the DUS test of the collection (Furones-Pérez and Fernández-López, 2009).

Phenology and size variables varied among years for the different environments, this lead to different rankings among years and sites and therefore should not be incorporated into classifications (dendrograms). From the dendrogram constructed with nine variables (Fig. 4), calculation of the average value for each cultivar and for both plantations resulted in 32 clusters, three composed of two cultivars. From the dendrogram constructed with the five variables (Fig. 5), calculation of the average value for each cultivar and for both plantations, resulted in 23 clusters, eight formed by two or more cultivars. However, otherwise undistinguishable cultivars were genetically distinguishable by use of nine enzyme systems (unpublished data). The dendrograms display great variation when more or less variables are incorporated, thus the relationships they establish between cultivars are not robust.

Comparison of the results *in situ* (Fernández-López, 1988-1991) and in collection characterizations was made with five common variables (Fernández-López, 1988-1991; Furones-Pérez and Fernández-López, 2009) that correspond to four morphological fruit descriptors (NutSplit, NutShape, HilumSize, HilumLW) and one morphological descriptor of the male flower (FilamentL). There are other common variables between *in situ* and in collection characterizations: the number of chestnuts per kilogram —NutSize—, the chestnut length —NutLength— and the number of embryos —Embryony— but they have bad properties such as descriptors for being subject to environmental variation by what they are excluded. The comparison was made separately for the two plantations: Agrovello (with 12 cultivars) and Sergude (with 35 cultivars) because the *ortets* used for each cultivar varied between plantations.

The five common variables used *in situ* and in collection characterizations are characteristics with good properties for the DUS test (Furones-Pérez and

Fernández-López, 2009). In spite of this, variations were identified in the states assigned in the *in situ* and collection evaluations. The characteristics with fewest differences were NutSplit and FilamentL. NutShape did not differ at Agrovello. Changes are important for HilumSize and HilumLW. Therefore, the large number of changes identified suggests once again that in accordance with UPOV requirements (1989), characterization must be performed in a collection and preferably with a minimum number of four replicates per genotype.

Acknowledgements

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