Clonal Forestry of chestnut in Northern Spain

Traditional *Castanea sativa* orchard

Clonal plantation of hybrids

Josefa Fernández-López, M. Eugenia Miranda-Fontaiña, Pilar Furones-Pérez, Beatriz Míguez-Soto
CINAM LOURIZÁN, XUNTA DE GALICIA, Apt. 127 36080 Pontevedra, Spain
ffina.cifal@siam-cma.org

VEGETATIVE PROPAGATION & DEPLOYMENT OF VARIETIES – THE SCOPE FOR EUROPE
LIVERPOOL 21, 22 & 23 April 2009

LOURIZÁN

XUNTA DE GALICIA
CONSELLERÍA DE MEDIO AMBIENTE E DESENVOLVEMENTO SOSTIBLE

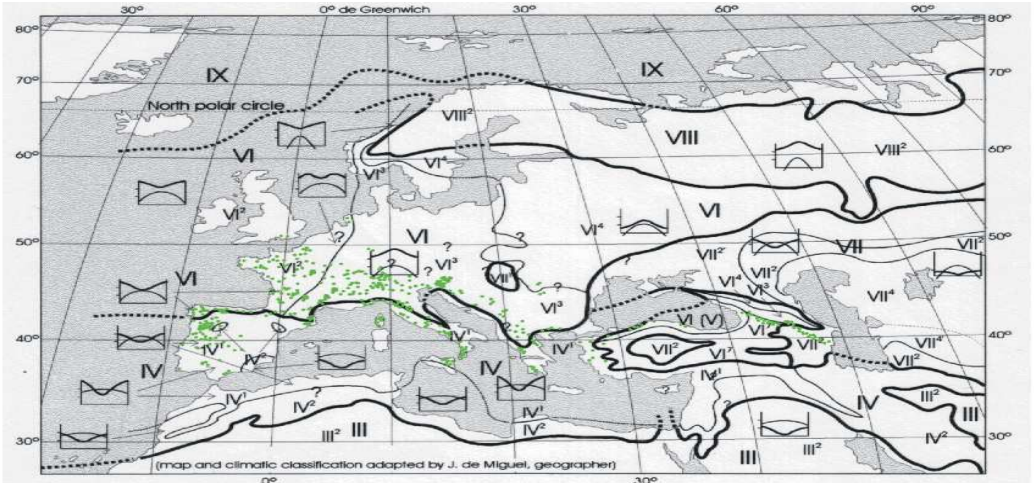
Treebreedex

Forest Research

•Clonal forestry of sweet chestnut has been developed during centuries in South European countries.

• By 1950 were initiated in Europe several breeding programmes of hybridization with asiatic chestnut species and in some countries was developed clonal forestry with hybrids.

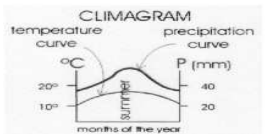
• Sweet chestnut is a circum-mediterraneam species with a southern limit caused by drought. It lives in permeable acid soils.



Climatic types	Subtypes	Climagram
III Desert subtropical	III² hot desert	
IV Mediterranean	IV¹ true	
	IV² subdesert	
V Atlantic	VI(V) temperated-humid	
	VI² oceanic	
	VI³ middle european	
	VI⁴ subboreal temperated	
	VI' sub-steppe	

Climatic types	Subtypes	Climagram
VII Steppe	VII² northern	
	VII⁴ southerndesert	
	VII'' northern semidesert	
	VII''' southern semidesert	
VIII Boreal		
IX Artic tundra and desert		

Chestnut distribution area



LOURIZÁN

- **Sweet chestnut is a multipurpose tree species managed for nut and wood production.**

- **Chestnut wood is characterized by the early heartwood formation which is conserved without chemical treatment.**

- **Ring shake is a wood problem very common in coppices but it is not important in plantations with other management.**

- **Chestnut is cultivated in orchards for nut or for nut and wood production. It is cultivated also in coppices. Now the doble aptitude option is still interesting although most plantations have as main objective wood production or nut production.**




LOURIZÁN

Traditional chestnut cultivars (*Castanea sativa* Miller) = Sweet chestnut

- **Are old selections, grafted, made by farmers by nut characteristics or wood quality.**
- **Are adapted to local environments**
- **It is neccessary to describe them for their inclusion in lists of commercial varieties.**
- **Traditional varieties for wood production to be used in plantations must be approved as Qualified or Controlled forest reproduction material**

LOURIZÁN

Breeding for resistance to *Phytophthora* and *Chryphonectria parasitica* in Europe was developed by 1950-1960 in Spain, Portugal, France and Switzerland.

- **Breeding in Spain was initiated by 1940 by Urquijo who made controlled crosses between sweet chestnut (*C. sativa*) and Japanese chestnut (*C. crenata).***
- **Further other collection was made by Vieitez who selected clones among seedlings obtained of F1 and F2 hybrids *C. crenata x C. sativa*.**
- **The main characteristic of this clones is the resistance to *Phytophthora* sp.**
- **Clones were propagated in nurseries by layering since 1960.**
- **Lourizán Research Centre initiated clonal selection in 1989.**
- **Hybrid clones to be used in forest plantations must be approved as Qualified or Controlled categories.**

LOURIZÁN

Regulations of chestnut reproduction materials

Directive 1999/105/CE commertialization of forest reproduction materials (FRM)

- Clones must be Qualified or Controlled
- Selected in field trials with appropiate design and analysis
- Candidates must be compared with CONTROLS/STANDARDS
- Identity of clones

General regulations about nursery plants and fruit trees

- List of comercial varieties / Conservation varieties
- Each variety must be DISTINCT, HOMOGE-NEUS, STABLE
- Description of the variety

LOURIZÁN

Tools and methodology for the selection of clonal varieties and their description (CINAM Lourizán)

1. Stablishment of Nuclear Clone Collections

2. Development of mass propagation methods

3. Identification of genotypes and their ancestry

4. Characterization: identification and selection

LOURIZÁN

Nuclear collections (Nuclear Stocks)
CLONE = ORTET + RAMETS

Mistakes in the identity of mother plants for the obtention of plants for field trials and for mass clonal propagation originate complete failure of clonal forestry.

Directive 1999/105/CE (FRM): All plants labelled as a clone must be originated from one unique plant (genet).

Propagules used as ortet must have juvenile characteristics

LOURIZÁN

Nuclear collections (Nuclear Stocks)

- The initial Nuclear Stock includes a number of ramets obtained from the ortet.
- The plants for clonal tests and for mother plants to be used in nurseries must belong to the same genet that the plants of the Initial Nuclear Stock.
- Molecular markers are the appropriate tool for this certification.
- If the methods of clonal propagation are in vitro then could be necessary to limit the number of subcultures to avoid somaclonal variation.

LOURIZÁN

Clonal propagation methods: axillary buds in vitro

In vitro clonal collection

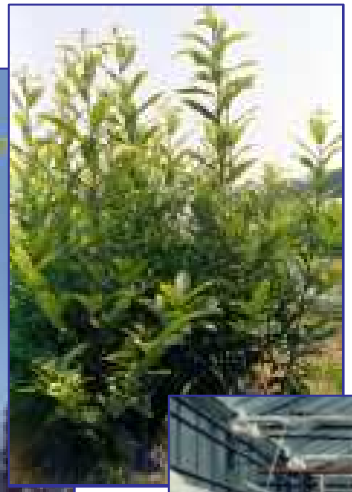
Stablishment

IN VITRO MULTIPLICATION OF CHESTNUT

In vitro multiplication

In vitro elongation

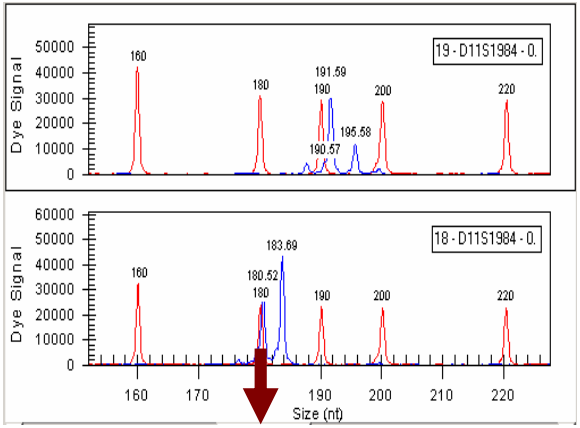
Nursery



Clonal Plantation

LOURIZÁN

Identification: Genetic markers for the identification of test and commercial Plants



	Isoenzymes	Microsatellites
Dominance	Codominant	Codominant
Variablility	Conservative	High mutación
Polimorfism	Low-Moderate	Very high
Laboratory cost	Cheap	Moderate-expensive
Reproducibility	Very high	High
Utilization	Identity/ancestry	Identity/ancestry

10 polymorphic loci

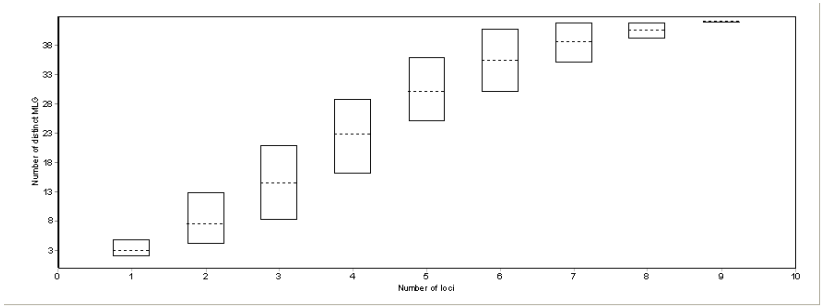
10 polymorphic loci

LOURIZÁN

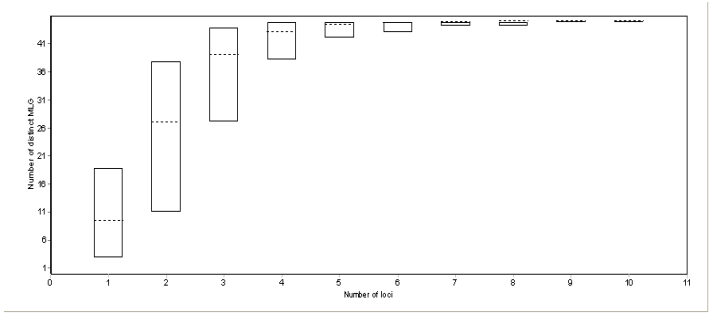
Identification of sweet chestnut cultivars

Number of loci required to identify 52 clones

Isozymes

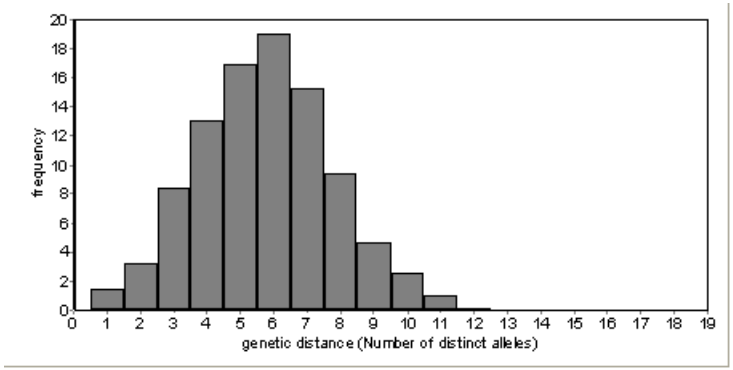


SSRs

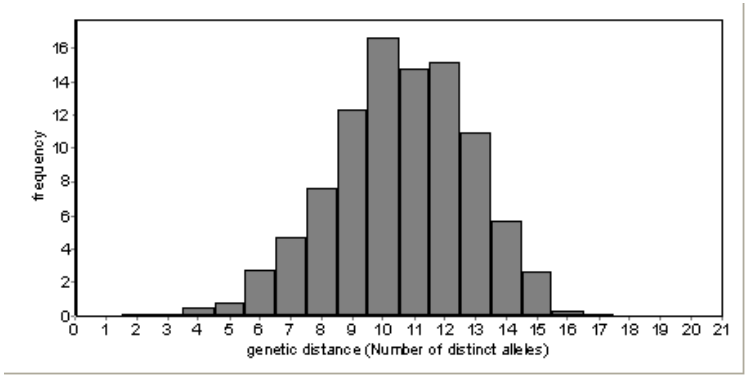


Distance between cultivars (Number of alleles)

Isozymes

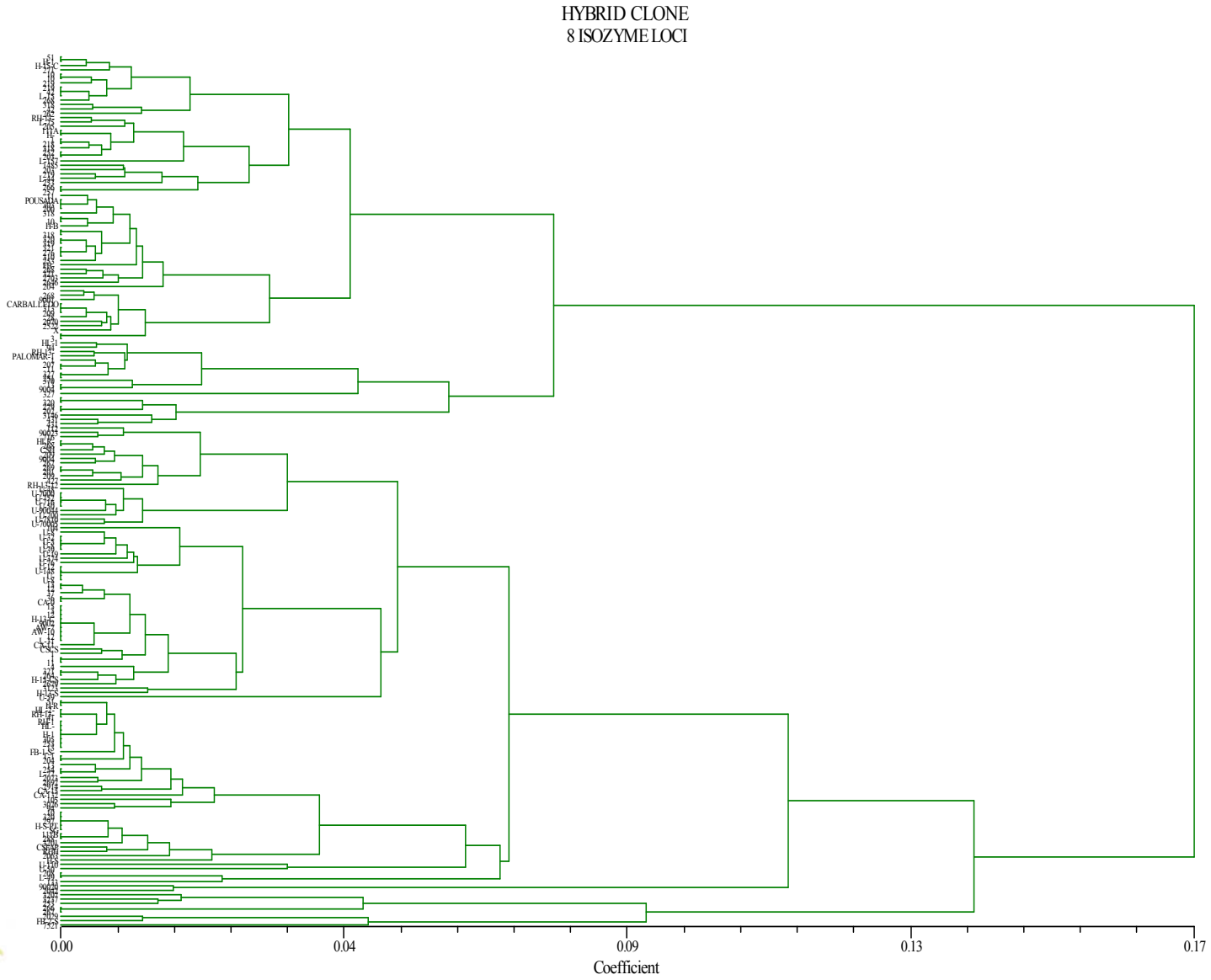


SSRs



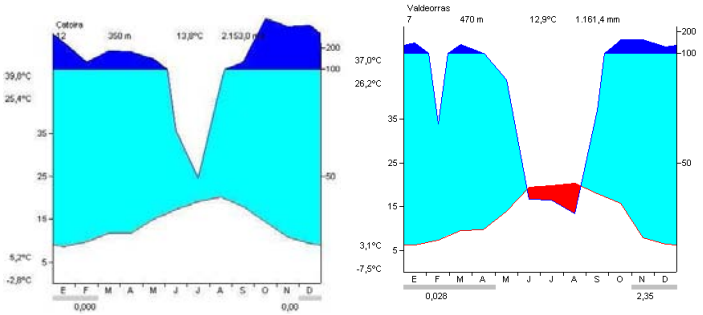
LOURIZÁN

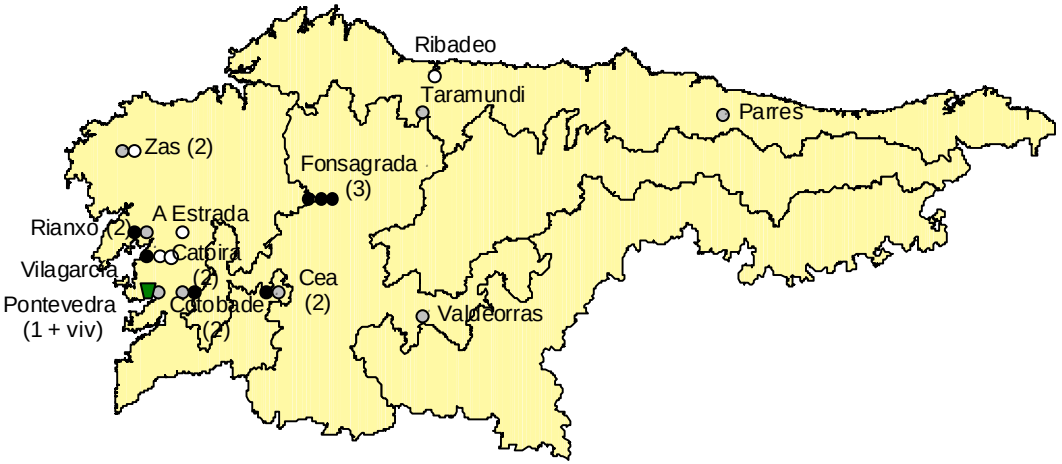
Identification of hybrid chestnut clone



LOURIZÁN

The network of field trials of chestnut hybrid clones in NW Spain





$$Xijk = \mu + Ci + Bj + \varepsilon k(ij)$$

$$H^2{}_C = \frac{\sigma^2{}_C}{\left(\sigma^2{}_C + \frac{\sigma^2{}_e}{k1}\right)}$$

$$r_{gAxy} = \frac{Cov_{g\,xy}}{\left(\sigma^2{}_{gx}\ \sigma^2{}_{g\,y}\right)^{1/2}}$$

Código	Sitio	Obj (1)	Fecha (2)	Prop (3)	Marco (4)	NPL (5)	NBL (6)	Parcela elemental (7)	NCLON (8)	NCTROL (9)
CHR_ECA1	Ribadeo	V, F, S	mar-89	A	4 x 4	350	2			4
CHR_ECA2	Catoira	V, F, S	mar-89	A	3 x 3	1133	3	5	80	8
CHR_ECA3	Zas	V, F, S	mar-89	A	3 x 4	1271	3	10	49	1
CHR_ECA4	A Estrada	V, F, S	mar-90	A	4 x 4,5	280	2			3
CHR_ECA5	Catoira	V, F, S	dic-94	A	3 x 3	693	3	5	45	1
CHR_ECIV1	Vilagarcía	V, F, S	dic-94	IV	3 x 4	1455	3	18	26	1
CHR_ECIV2	Fonsagrada D	V, F, S	mar-98	IV	3 x 3	172	69-87	1	9	1
CHR_ECIV3	Fonsagrada A	V, F, S	mar-98	IV	3 x 3	498	50	1	11	1
CHR_ECIV4	Fonsagrada C	V, F, S	mar-98	IV	3 x 3	193	51-69	1	10	1
CHR_ECIV5	Cotobade	V, F, S	feb-03	IV	3 x 3	190	1-10	1	25	2
CHR_ECIV6	Cea	V, F, S	mar-04	IV	3 x 3	164	11-20	1	17	1
CHR_ECIV7	Rianxo	V, F, S	mar-05	IV	3 x 3	720	40			
CHR_ECE1	Zas	V, F, S	feb-99	E	5 x 5	624	30	1-2-3	13	0
CHR_ECE2	Valdeorras	V, F, S	mar-00	E	3 x 3	435	13	1	38	1
CHR_ECE3	Taramundi	V, F, S	abr-02	E	3 x 3	367	20	1	27	1
CHR_ECE4	Parres	V, F, S	abr-02	E	3 x 3	683	20	1	57	16
CHR_ECE5	Cotobade	V, F, S	feb-03	E	3 x 3	302	11-20	1	45	3
CHR_ECE6	Pontevedra	V, F, S	feb-04	E	3 x 3	222	10	1	30	1
CHR_ECE7	Cea	V, F, S	mar-04	E	3 x 3	241	1-10	1	35	1
CHR_ECE8	Rianxo	V, F, S	mar-05	E	3 x 3	1153	40	1	53	1
CRH_EV1	Pontevedra	V, F, S	feb-99	E		1357	40	1	55	1
CRH_EV2	Pontevedra	V, F, S	feb-05	E		612	20	1	32	1

LOURIZÁN

Types of traits for the characterization of chestnut clones

IDENTIFICATION TRAITS

•Directive 1999/105/CE: Clones must be identified by their distinctive traits (morphologic or behavioral traits).

• UPOV descriptors can be used: UPOV (1989) Guidelines for the conduct of tests for distinctness, homogeneity and stability. Chestnut (*Castanea sativa* Mill.). TG/124/3 International Union for the Protection of New Varieties of Plants.

SELECTION TRAITS

•Obligatory in Directive 1999/105/CE: adaptation, wood production, wood quality, stem straightness.

•Additional traits for chestnut: resistance to Phytophthora and nut quality.

LOURIZÁN

Description of varieties: The use of the descriptor method

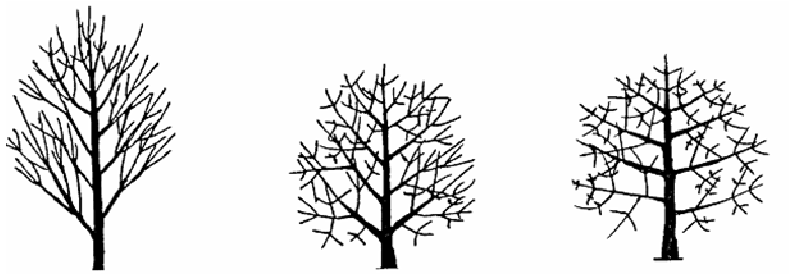
A descriptor is a characteristic or attribute that refers to the form, structure or behavoiur of an accession in a germplasm collection

A DESCRIPTOR has a NAME, SEVERAL STATES, A NUMERIC CODE FOR EACH STATE

NAME: Flushing date (TG/124/3)

STATE	DATE	NOTE
Very early	>25 March	1
Early	26 March-15 April	3
Medium	16 April – 30 April	5
Late	1.15 May	7
Very late	> 15 May	9

NAME: Growth habit (TG/124/3)



1 2 3

STATE	NOTE
Erect	1
Semierect	5
Spread	7

LOURIZÁN

Use of the descriptor method for the characterization of clones with data obtanined from field trials or nursery trials

$$X_{ijk} = \mu + Site_i + Clon_j + Site \times Clon_{ij} + e_{ijk}$$

The properties of each traits as descriptor will be estimated:

- **Distinctness: demonstrate clear differences between clones included in a test**

$$H^2_C = \frac{\sigma^2_C}{k_1\sigma^2_\varepsilon + \sigma^2_C + k_2\sigma^2_{PC}}$$

- **Uniformity: intra-clon homogeneity**

- **Stability: temporal or spatial variation**

$$pl = \frac{\sigma^2_P + \sigma^2_{PC}}{\sigma^2_\varepsilon + \sigma^2_P + \sigma^2_C + \sigma^2_{PC}}$$

LOURIZÁN

Criterium for judging a descriptor evaluated in a clonal test

	Distinctness	*Uniformity*	*Stability*
	Estimators used		
	H^2_C	**Error**	**PI**
[0.00, 0.20]	**Very low**	**Very high**	**Very high**
[0.21, 0.40]	**low**	**high**	**high**
[0.41, 0.60]	**medium**	**medium**	**medium**
[0.61, 0.80]	**high**	**low**	**low**
[0.81, 1.00]	**Very high**	**Very low**	**Very low**

LOURIZÁN

Properties of several traits evaluated in a clonal test as descriptors

Variable	Distinction	Uniformity	Stability
Growth habit	High	Low	Very high
Branch angle	High	Medium	Very high
Branch density	High	Medium	Very high
Height	Very low	Medium	High
BudBurst	Very high	Very high	medium-high
FilamentL	Very high	High	Very high

LOURIZÁN

Description of sweet chestnut clones after evaluation in two field trials

VARIETY	Growth habit	Branch angle	Branch density
Loura	Erect	Acute	reduced
Famosa 1	Erect	Acute	very redu.
Presa	Erect	Acute	reduced
Raigona	Erect	Right	moderate
Ventura	Erect	Acute	moderate
Negral 1	Erect	Right	moderate
Amarelante 1	Erect	Right	reduced
Blanca 1	Semierect	Obtuse	moderate
Inxerta	Semierect	Right	moderate
Longal	Semierect	Right	reduced
Rapada 1	Semierect	Right	high
Parede 1	Semierect	Right	high
Verde 1	Semierect	Obtuse	reduced
Luguesa	Sparce	Obtuse	abundant

LOURIZÁN

RECOMENDATIONS ABOUT CHESTNUT CLONAL FORESTRY

- **Promotion of traditional cultivars in the traditional areas in plantations with objectives 'nut and wood'.**
- **Promotion of hybrid clones in Atlantic climate, in excelent soils if the objective of plantations is 'wood production'**
- **Practical development of clonal forestry before the selection is completed damages the image of breeding.**
- **The costs of clonal plants is 3 to 5 times the price of seedlings. To reduce costs it is possible to develope family forestry and limit clonal forestry to most productive clones and sites.**
- **Directive 1999/105/CE (FRM) gives a lot of criteria but don´t say enough about how to do selection, how to demonstrate the clonal fidelity or how to describe the selected varieties. For this purposes it is necessary to develop technical guideliness.**
- **Is there a reason to limit the number of subcultures in vitro through processes of multiplication of clones?**

LOURIZÁN