Genetic variation and genotype-environment interactions in short rotation *Populus* plantations in southern Europe

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Abstract Growth and production in the first year, as additional selection criteria, were assessed for nine poplar clones to be used as short rotation woody crops (SRWC) in the production of biomass for energy purposes. In order to identify the most promising clones in terms of growth and yield and also to assess their stability, trials were established at different locations in Spain. The majority of these clones, which form part of the European list of base materials, have frequently been used in plantations aimed at timber production but not for biomass in Mediterranean conditions. Others, such as those selected in Italy specifically for biomass production (currently provisionally admitted), are being tested for the first time under different soil and climatic conditions in Southwest Europe. The early selection of clones for rapid juvenile growth provides a valuable additional input to the clonal selection process, especially where very short rotations are desired (no more than 3 years). In any case, determining clonal stability in terms of growth is of great use not only when deciding on the clones to be used in plantations but also when developing breeding programs. ANOVA and Genotype plus Genotype × Environment (GGE) biplot analyses were used to analyse the growth and stability of the clones, which were then ranked according to mean performance and stability. Differences were detected between clones as well as between the different environments tested. The biplot analysis allowed different groups of clones to be identified according to their performance and degree of interaction displayed, thus providing useful information for the selection process. The production of aboveground biomass in the first vegetative period ranged from 1.7 to

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8.0 Mg DM ha^{-1} at the different sites. 'Monviso', 'Guardi', 'AF2' and '2000 verde' were the most productive clones whereas 'Unal', 'Pegaso' and 'USA 49-177' were the least productive. The stability analysis identified 'AF2', 'Guardi', 'I-214' and 'MC' as more stable clones while 'Monviso', '2000 verde', 'Unal', 'Pegaso' and 'USA 49-177' were found to be specifically adapted to certain environments. This implies that where information on site conditions is not available, the 'AF2' and 'Guardi' clones offer greater assurance of successful establishment and higher initial growth. The growth of 'Monviso' '2000 verde' 'Unal' 'Pegaso' and 'USA 49-177' clones is highly dependent on site conditions during the establishment phase. Similarly, the SH (Shore Henares river) and LT (La Tallada) sites were identified as the most highly discriminative environments for the set of clones while CS (Cubo de la Solana) and AR (Atarfe) were identified as those where performance levels were average.

Introduction

The need to increase the number of renewable energy sources in the context of the changing energy model in Europe has led to the consideration of biomass (PFER 2004) and more specifically, the production of lignocellulosic biomass through Short Rotation Forestry (SRF) as a viable alternative. It would also contribute towards the objectives outlined in the Renewable Energy Action Plan (EU 2009).

The European Environmental Agency (2008) has estimated at around 19.3 Mha the total amount of land available for energy crops in the European Union by 2030, with Spain potentially contributing around 2.5 Mha to this total. At present, the figures for Spain are considered to be slightly higher, at around 3.9 Mha with an expected production of around 41 Mt of dry matter (DM) from lignocellulosic biomass and an energetic potential of 16 Mtep (Fernandez 2009).

Poplar is one of the most promising forest species for SRF in the Mediterranean countries of Europe (Paris et al. 2005), displaying many of the appropriate characteristics for this purpose such as rapid growth, adaptability to high density, high photosynthetic capacity and suitability for coppicing (Ceulemans et al. 1996). Additionally, poplar has long been cultivated throughout much of Europe for timber production, so a substantial knowledge base exists with regard to its behaviour and management. The use of poplar in SRF to produce biomass for energy purposes could provide a valuable alternative for farmlands faced with abandonment due to European agricultural reforms and the impact of the latter on the development of rural areas (Bonari and Pámpana 2002). In addition, significant environmental benefits (i.e., reducing mineral depletion or reducing net CO_2 in the atmosphere) and social benefits (less interference with food market) are achieved with this type of crop in comparison to traditional crops.

Although a lot of information is already available with regard to this kind of plantation (Ceulemans and Deraedt 1999; Tharakan et al. 2001; Tullus et al. 2009, among others), further research is required into management related aspects such as densities, harvesting cycles or cultural practises, many of which are often linked to or conditioned by harvesting logistics or local conditions (Sixto et al. 2007a). Other aspects relating to the genetic material also require greater understanding such as efficiency in the use of resources (Monclus et al. 2009), suitability for coppicing (Laureysens et al. 2005) or response to pests and diseases (Broderick et al. 2010). Moreover, factors related to potential site

productivity and the adaptation of the material to each site with the aim of improving production while fulfilling sustainability criteria must be carefully assessed in order to optimise the economic viability of the plantations. In this respect, the above-mentioned factors can lead to great variations in poplar biomass yield, with productions ranging from 20–25 t DM ha^{-1} year^{-1} (Scarascia-Mugnozza et al. 1997; Mareschi et al. 2005) for the South of Europe, to 8–12 t DM ha^{-1} year^{-1} for North and central Europe (Makeschin 1999; Ericsson et al. 2009).

In the context of the current demand for information regarding the suitability of genetic material for use in SRF for biomass production, an early evaluation of the growth and yield of potentially viable clones is essential to identify their potential productivity (Kumar and Singh 2001). The evaluation of different growth traits has proved to be effective for early clone selection in many fast-growing species such as *Populus* sp. (Kumar and Singh 2001), *Pinus radiata* D. Don (Li and Wu 2005) or *Eucalyptus* sp. (Marques et al. 1996) and has also been used successfully in mid-rotation selection, for example, in the case of eucalyptus (Blake et al. 1990) or poplar (Demeritt 1979). In SRF, a high correlation between the initial and final response of the clones implies greater reliability and usefulness of early phenotypic selection (Rae et al. 2004) and despite the possible genotype × time interaction, early selection would seem appropriate to hasten the commercial use of clones which display an initial superiority, especially where short term responses are required. In any case, by assessing the growth response of the clones during the establishment phase, adaptive advantages can be identified, thus providing an additional criterion for selection (Stanton 2001). The availability of information regarding growth and production in the first year is also useful for evaluating the genotype growth patterns (Tharakan et al. 1998).

Given the importance of the genotype × environment interaction (Strobl 1988), it is necessary to determine the stability of the response, determining the behaviour of the clones in the different areas selected for cultivation. Phenotypic plasticity is understood as the ability of a genotype to produce different phenotypes in response to differences in environmental conditions (West-Eberhard 2003). Sultan (1995) distinguishes between passive or inevitable plasticity, observable through describing the morphological and/or physiological type changes, as opposed to adaptive phenotypic plasticity which has to do with the potential adaptive value of these changes.

In addition to determining the behaviour of the clones in terms of growth and yield, it is important to assess the stability of the clones in relation to these traits in different environments, in other words, to ascertain the importance of the genotype × environment interaction in each case. By furthering our understanding of clonal behaviour, not only in terms of production but also stability, more appropriate choices can be made as regards the material employed and thus, the likelihood of success in the improvement programmes is enhanced. When assessing the profitability of a plantation, the choice of material is greatly facilitated if the grower possesses information regarding the stability of the clones. Although both of these aspects are commonly combined in the sphere of agriculture, it is unusual in studies concerning forest crops.

A number of statistical procedures are available for analyzing the information gathered from the trials established in different environments. The biplot technique (Gabriel 1971) allows the main genotype effect plus genopyte × environment interaction (GGE) to be visualized using data from multi-environment trials, and is therefore ideal for analyzing trials of this type (Yan and Kang 2002). Although rarely used in the analysis of forestry data (Ding and Yan 2007), the biplot technique (GGE) provides a graphical analysis of the genotype behaviour and the genotype × environment interaction, especially when this interaction constitutes the most significant source of variation (Ibáñez et al. 2006).

The aim of this study, therefore, is to evaluate the differences in the growth and yield of different poplar hybrid clones (including clones especially selected for biomass production) at an early stage (first growth period) in different environments, in order to identify the clones which display adaptive advantages in terms of both production and response stability during the crop establishment phase. This information could be particularly useful in improvement programmes aimed at obtaining varieties which are highly productive over very short timescales.

Materials and methods

Plant material

Nine clones were included in the trials, six of which form part of the National Catalogue of Basic Materials for the *Populus* genus having been commonly used for timber production in this geographic area although not for biomass production. The others were recently included in the Italian Catalogue, having been specifically selected for biomass production although no information is available as regards their performance in other areas with Mediterranean or continental climates.

These clones belong to different interspecific hybrids of *Populus*: five *P. × canadensis* Moench (*P. deltoides* March. × *P. nigra* L.); two *P. × generosa* Henry (*P. trichocarpa* T.&G. × *P. deltoides*) and two ([*P. deltoides* × *P. trichocarpa*] × *P. nigra*). Clone 'I-214' was used as a control because of its widespread cultivation in the study area. Information regarding the clones is detailed in Table 1.

Table 1 Name, parentage, origin and sex of the clones included in the multi-site plantations

Clone name	Parentage	Origin	Sex	Additional information
I-214	*P. deltoides* × *P. nigra*	Italy	F	Clone included in the Spanish catalogue in 1992 but not specifically for biomass
MC	*P. deltoides* × *P. nigra*	Italy	F	Clone included in the Spanish catalogue in 1992 but not specifically for biomass
2000 verde	*P. deltoides* × *P. nigra*	Italy	M	Clone included in the Spanish catalogue in 2003 but not specifically for biomass
Guardi	*P. deltoides* × *P. nigra*	Italy	F	Clone included in the Spanish catalogue in 2003 but not specifically for biomass
AF2	*P. deltoides* × *P. nigra*	Italy	M	Provisional acceptance in the Italian catalogue. Selected specifically for biomass
Unal	*P. trichocarpa* × *P. deltoides*	Belgium	M	Clone included in the Spanish catalogue in 2003 but not specifically for biomass
USA49-177	*P. trichocarpa* × *P. deltoides*	USA	F	Clone included in the Spanish catalogue in 2003 but not specifically for biomass
Monviso	(*P. deltoides* × *P. trichocarpa*) × *P. nigra*	Italy	F	Provisional acceptance in the Italian catalogue. Selected specifically for biomass
Pegaso	(*P. deltoides* × *P. trichocarpa*) × *P. nigra*	Italy	M	Provisional acceptance in the Italian catalogue. Selected specifically for biomass

F female, *M* male

Trial sites and experimental design

Five trials were established in spring using similar planting and maintenance procedures at each site. Hardwood cuttings of 20–30 cm in length previously soaked in water for 48 h, were planted by hand.

The plantation density was of 33.333 cuttings·ha^{-1} (spaced 1 × 0.30 m). Although trials at high densities are more common in the case of willow (for example 40,000 stems/ha in Stolarski et al. 2008) than in the case of poplar, we opted for a very high density plantation and short rotations (2–3 years) in order to determine the potential of the crop at this density.

The whole trial area at every site (2.500 m^2) was fertilized when the soil was tilled using a NPK fertilizer (8–15–15) at 600 kg ha^{-1}. Immediately after the cuttings had been planted, oxifluorfen (4 l ha^{-1}) was applied over the whole area in order to control the weeds during the first months of growth until the plantations were established. Irrigation was applied according to the requirements at each site.

The five experimental sites, which are referred to using the name of the nearest village or geographical feature, are as follows: (Fig. 1): Shores of the Henares river (SH), Cubo de la Solana (CS), Atarfe (AR), La Tallada (LT) and Villafer (VF). Coordinates, climatic and edaphic characteristics at the different sites are shown in Table 2. Edaphic characterization was made down to 20 cm because the drip irrigation system favoured rooting in this area.

A multi-environment trial with a split-plot design was employed. Site (five levels) was the main factor with four blocks and three replications per block at each site; clone was the secondary factor (nine levels), so 540 plots were stablished in total. Each plot comprised a total of 25 ramets per clone and site but only 9 were evaluated in order to avoid the border effect. Hence, a total of 4.860 were evaluated.

Several variables were measured during as well as outside the vegetative period to assess the performance of the clone at each site: diameter (in mm) over bark at 10 cm (d_{10}) and 1.30 m (dbh), measured using a digital calliper; total height (in cm) measured using a pole (HT). Production was measured by recording aboveground total dry biomass
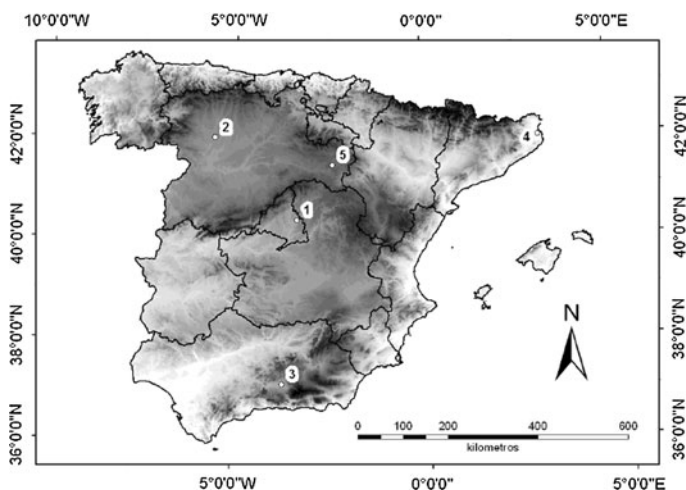

Fig. 1 Location of the experimental sites: *1* Shores of the Henares river (*SH*), *2* Villafer (*VF*), *3* Atarfe (*AR*), *4* La Tallada (*LT*) and *5* Cubo de la Solana (*CS*)

Table 2 Summary of soil (top 20 cm) and climatic attributes in the study site locations

Sites	Coordinates		Altitude	AR	T	Max T	Min T	EC	pH	Clay	Lime	Sand	OM	K
	Latitude	Longitude	m asl	(mm)	(°C)	(°C)	(°C)	Mmhos/cm		(%)	(%)	(%)	(%)	(%)
LT	42.05	2.91	20	703	15.7	29.2	3.4	0.28	8.3	8.9	26.2	65.0	0.95	19.0
AR	37.13	3.41	590	429	16.5	34.6	3.7	0.38	8.5	21.8	17.3	60.9	0.94	243.3
VF	42.07	5.45	730	499	11.7	29.6	−0.9	0.18	8.3	16.8	10.3	72.9	1.04	214.7
SH	40.28	3.22	595	464	13.8	33.8	0	0.23	8.4	15.8	15.3	68.9	0.49	248.6
CS	41.36	2.3	1090	616	9.7	27.4	−2.5	0.07	6.8	8.2	5.6	86.2	0.6	61.3

asl above sea level, *AR* annual rainfall, *T* average annual temperature, *Max T* hottest month average maximum temperature, *Min T* coldest month average minimum temperature, *EC* electrical conductivity, *OM* organic matter, *K* potassium

Shores of the Henares river (*SH*), Cubo de la Solana (*CS*), Atarfe (*AR*), La Tallada (*LT*) and Villafer (*VF*)

(Mg DM ha^{-1}—BT), estimating the dry weight of a subsample (oven-dried to constant weight at 100°C) taken from the nine plants contained in 12 plots per clone and site. Stem and branch biomass were weighed separately and the stem/branch biomass ratio (S/B ratio) was used to assess the biomass distribution in the different clones.

Statistical analysis

A multifactor analyses of variance (ANOVA) was performed for each response variable (d_{10}, dbh, HT and BT) to test the overall effect. The standard model for the experimental design is expressed as follows:

$$y_{ijk} = \mu + \delta_i + \alpha_j + (\delta\alpha)_{ij} + \beta_k + (\alpha\beta)_{jk} + e_{ijk} \tag{1}$$

where y_{ijk} is the observed value for the response variable (diameter, height or biomass) at site j, clone k and in the block i; μ is the overall mean, δ_I, α_j and β_k are the block, site and clone effects respectively; $(\delta\alpha)_{ij}$ and $(\alpha\beta)_{jk}$ are the bifactoral interactions and e_{ijk} is the experimental error, which is in fact equal to $(\delta\beta)_{ik} + (\delta\alpha\beta)_{ijk}$.

Where a significant treatment effect was observed ($P \leq 0.05$), a multiple comparison between the different levels was performed to arrange the clones into groups according to the similarity of the response variable using Tukey's mean test. Models were fitted and tests performed using the SAS statistical package (SAS 2004).

To interpret the genotype × environment interaction, an analysis was performed based on the graph created using the biplot (symmetrical scaling) (Yan 2002; Ding and Yan 2007). To explore the variability between genotype plus genotype × environment (GGE) in biomass production, a SREG (site regression) linear-bilinear model was used, given by:

$$Y_{ij} = \mu + \beta_j + \left(\sum_{n=1}^{k} \lambda_n \cdot \xi_{in} \cdot \eta_{in}\right) + \varepsilon_{ij} \tag{2}$$

where Y_{ij} is the clone response (biomass production) in environment j; μ is the overall mean; β_j is the environment effect; λ_n is the eigen value; ξ_{in} and η_{jn} are, respectively, the coefficients of clones and environments for n = 1,2,…, (termed primary and secondary purpose of clone and environments respectively), and ε_{ij} is the random error term, which is assumed to be normal and independently distributed (0, s^2/r) (where s^2 is the error set variance and r is the number of replicates). The GGE biplot was based on the symmetrical scaling of the first two principal components (PC1 and PC2) resulting from the analysis of PC data focusing on environment (Yan and Kang 2002). Models were fitted and tests performed using the SAS statistical package (SAS Institute Inc 2004).

Results

Growth and production

Significant differences were found among clones for all the growth (d_{10}, dbh, HT) and production variables analysed (BT and S/B ratio), as well as for sites and the interaction between clone and site (Table 3).

The data recorded for the different clones reveals that the greatest height growth corresponds to clones 'AF2', 'Monviso', '2000 verde' and 'Guardi' whereas the poorest

Table 3 The effect of clone and site on different response variables according to the analysis of variance ($P < 0.001$) using value of F test

Sources	HT		d_{10}		dbh		BT		S/B ratio	
	F	df	F	df	F	df	F	df	F	df
Clone	24.16	8	45.66	8	11.15	8	11.01	8	6.76	8
Site	394.54	4	372.27	4	570.39	4	264.55	4	14.53	3
Clone × site	6.65	32	9.07	32	45.66	32	5.70	32	2.69	24

HT total height in cm, d_{10} diameter at 10 cm of soil in mm, *dbh* diameter at 1.30 m in mm, *BT* total dry biomass in Mg DM ha^{-1} year^{-1}, *S/B ratio* ratio of stem and branch biomass, *F* value of *F* test, *df* freedom degree

Table 4 Response of the clones for the different growth and production variables

Clones	HT	d_{10}	dbh	BT	S/B ratio
'2000 Verde'	242 a	17.2 bc	11.3 bc	4404 ab	6.67 b
'AF2'	238 a	18.9 a	12.8 a	4507 ab	26.23 a
'Guardi'	252 a	17.7 bc	11.5 b	4562 ab	12.67 b
'I-214'	221 b	18.0 a	10.2 d	4060 bc	11.55 b
'MC'	220 b	17.9 b	11.1 c	3899 bc	35.16 a
'Monviso'	242 a	17.8 bc	12.3 ab	5324 a	8.52 b
'Pegaso'	207 cd	15.8 d	10.3 d	3339 c	26.91 a
'USA 49-177'	215 cd	16.8 cd	10.1 d	3952 bc	6.18 b
'Unal'	201 d	14.9 e	8.8 e	3423 c	8.17 b

Mean value

HT total height in cm, d_{10} diameter at 10 cm of soil in mm, *dbh* diameter at 1.30 m in mm, *BT* total dry biomass in Mg DM ha^{-1}year^{-1}, *S/B ratio* ratio of stem and branch biomass

Significant differences are denoted by lowercase letters ($P = 0.0001$) according to Tukey's mean test

results are displayed by 'Unal', 'USA 49-177' and 'Pegaso' (Table 4). When the variable analyzed is the diameter (d_{10} and dbh), the ranking of the clones is very similar, taking into account that the correlation coefficient between these variables indicates a fairly strong relationship (0.84). In addition, Sixto et al. (2007c) found that the correlation between the diameter and the biomass in the first year of growth differed from one clone to another. Greater correlation was observed in the previous work between biomass and diameter at 1.30 cm in the case of the most productive clones and between biomass and diameter at base for the least productive. Hence, these two diameter variables are those considered in this study.

The clones with the greatest total biomass production are also 'Monviso', 'Guardi', 'AF2' and '2000 verde' and the lowest production corresponds to 'Unal' and 'Pegaso' (Table 4). The stem/branch ratio also differed significantly ($P < 0.001$) among clones (Table 3), with 'Pegaso', 'AF2' and 'MC' exhibiting the least amount of branch biomass.

The ranking for clone growth at each site (Table 5) is expressed as a percentage of the control clone 'I-214' values for each site. The variable dbh^2 × HT (Sixto et al. 2007b) was employed in the evaluation of volume per tree. Certain clones, such as 'Monviso' or 'AF2' were generally the most productive. Others, such as 'Guardi' occupy a similar position in the ranking at all the sites, suggesting higher stability. The worst performance at all sites was exhibited by the 'Unal' 'Pegaso' and 'USA 49-177' clones.

Table 5 Clone ranking (R) in the different sites expressed as a percentage of I-214 (*in bold*) clone using dbh^2 × HT for growth evaluation

Sites									
SH		VF		AR		LT		CS	
Clone	R	Clone	R	Clone	R	Clone	R	Clone	R
'2000 verde'	165	'Monviso'	223	'AF2'	329	'Monviso'	276	'Monviso'	332
'AF2'	157	'AF2'	181	'Guardi'	176	'AF2'	242	'2000 verde'	323
'MC'	122	'Guardi'	174	'MC'	131	'USA 49-177'	213	'Guardi'	261
'Guardi'	110	'Pegaso'	123	'Monviso'	105	'Guardi'	163	'USA 49-177'	254
'Monviso'	108	**'I-214'**	**100**	'2000 verde'	101	'2000 verde'	144	'Pegaso'	215
'I-214'	**100**	'MC'	89	**'I-214'**	**100**	'MC'	118	'AF2'	210
'Pegaso'	78	'2000 verde'	87	'Unal'	96	**'I-214'**	**100**	'MC'	184
'Unal'	65	'USA 49-177'	73	'USA 49-177'	59	'Pegaso'	65	'Unal'	163
'USA 49-177'	44	'Unal'	21	'Pegaso'	32	'Unal'	52	**'I-214'**	**100**

Shores of the Henares river (*SH*), Cubo de la Solana (*CS*), Atarfe (*AR*), La Tallada (*LT*) and Villafer (*VF*)

The productivity observed in the different trials in terms of growth and yield is presented in Fig. 2. The highest value was obtained at the SH site and the lowest at the AR site.

Genotype-site interaction

The significant interaction detected between clones and environment indicates that clone response varies according to site characteristics. The genotype and genotype × environment interaction for biomass production, represented by the biplot GGE, explains 92.96% of the variation, of which 53.05% corresponds to principal component 1 (PC1), and 33.91% to principal component 2 (PC2) (Fig. 3).

The biplot enables visual comparison of the clones, sites and their interrelationships. Each clone and environment in the GGE biplot is represented by a marker (rhombus and circles respectively). The clone markers furthest away in bi-dimensional space ('2000 verde', 'Monviso', 'USA 49-177', 'Unal' and 'Pegaso') are connected by straight lines, thus shaping the vertices of the polygon. These are the clones which show the most interaction (more unstable or plastic among sites), being the most and least productive. The clones on the vertex of the polygon situated within each of the sectors are those which display the greatest productivity for the environments included in the same sector. It can be seen, therefore, that the 'Monviso' clones displays a proportionately higher performance compared to the rest of the clones tested at the LT and VF sites and that '2000 verde' interacted very positively with the SH site, performing very well in that environment. However, an adverse interaction is evident for the same clone '2000 verde' with the LT site, where biomass yields are very low. Furthermore, the 'I-214', 'MC', 'Guardi' and 'AF2' clones displayed little interaction with any of the environments tested, although the 'Guardi' clones showed greater productivity at the AR and VF sites, as did 'AF2' at the AR site (Fig. 3 upper right). 'Unal' and 'Pegaso' exhibited adverse interaction, that is, their biomass production was lower for all environments, and was proportionately lower at the LT, VF and AR sites (as reflected by the increased distance between the representation of the clones and sites). Similarly, 'USA 49-177' also showed proportionately lower yields

Fig. 2 Clone response to site for the different growth and production variables. **a** total height; **b** diameter; **c** total biomass; **d** S/B ratio. Mean values are presented. For each variable and site, significant differences are denoted by different lowercase letters ($p=0.0001$) according to Tukey's mean test. Shores of the Henares river (*SH*), Cubo de la Solana (*CS*), Atarfe (*AR*), La Tallada (*LT*) and Villafer (*VF*). Stems and branches were not sampled separately at *LT*
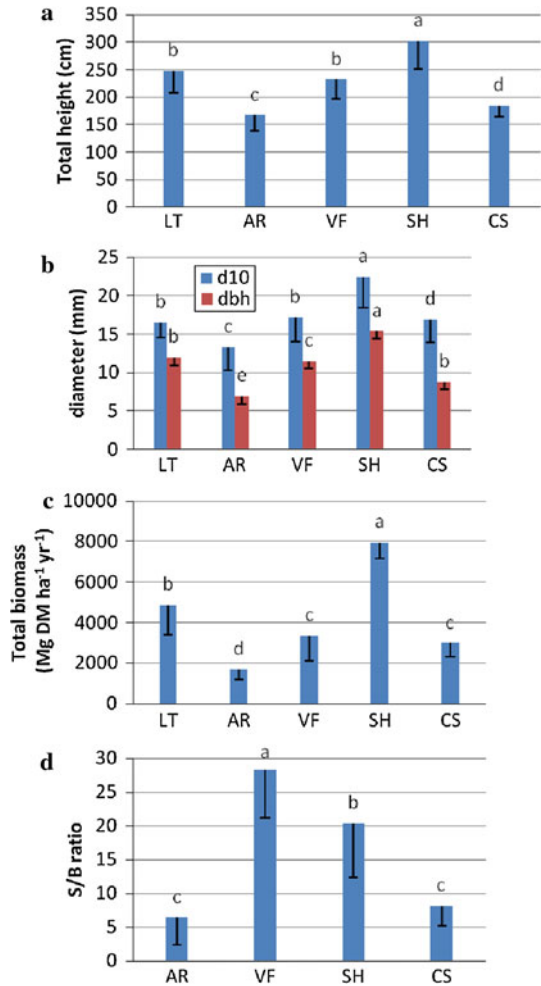
compared to the rest of the clones at the SH site. As regards to the environments, the LT and SH sites were the most interactive and CS was the least.

Discussion and conclusions

The mean production of aboveground biomass in the first vegetative period at the different locations included in our study ranged from 1.7 to 8.0 Mg DM ha^{-1} year^{-1}. Bearing in mind that during this first year, a significant quantity of the biomass produced by the plant is employed in the establishment of the root system (Pregitzer et al. 1990; Wullschleger et al. 2005), this figure can be considered high. If the complete crop cycle is considered, according to Makeschin (1999), once the aerial biomass gains importance, production ranges from 1.2 to 12 Mg DM ha^{-1} year^{-1}.

The productivity observed at the different trials was highest at the SH site and lowest at the AR site (Fig. 2). Although the AR site (1.7 Mg DM ha^{-1} year^{-1}) has the longest
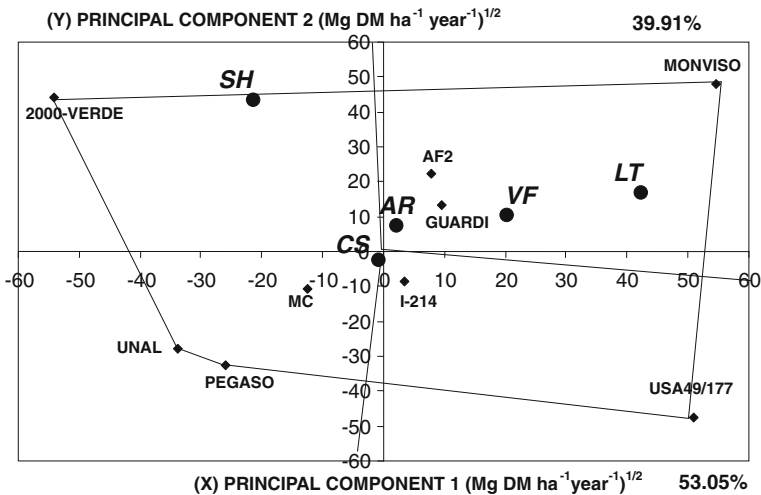
Fig. 3 Genotype plus genotype × environment (*GGE*) biplot obtained from yield in nine clones and five environments to explore the variability in biomass production. Shores of the Henares river (*SH*), Cubo de la Solana (*CS*), Atarfe (*AR*), La Tallada (*LT*) and Villafer (*VF*)

vegetative period and warmest temperatures (and therefore, in theory, the greatest site potential), watering restrictions, which coincided with the end of the vegetative period, along with deficient weed control, probably due to a lower effectiveness of the herbicide and the existence of a larger seed bank in the soil, led to a loss in productivity. However, these results should not be regarded as exceptional since watering restrictions are likely to be imposed again in the future; hence we consider this situation reflects a real scenario in the Mediterranean area.

Regarding the clones used in the trials, productivity varied from 3.3 Mg DM ha^{-1} year^{-1} for the 'Pegaso' clone to 5.3 Mg DM ha^{-1} year^{-1} for the 'Monviso' clone, which showed the highest production. These results are consistent with those of a study developed in Northern Italy (Nardin and Alasia 2004).

A number of authors have reported a lack of significant correlation between the growth and yield observed at the beginning and at the end of the rotation; particularly where the rotation period considered is longer (Demeritt 1981) although this situation has also been observed in shorter rotations (Marron et al. 2006). Nevertheless, in the case of short rotation systems, early phenotypic characterisation is useful as an additional selection criterion (Stettler et al. 1992), especially for rotations of 2–3 years in length. This early response of the vegetal material, as well as its interaction with the site, provides very useful information which can be used to achieve successful plantations by allowing clone sets to be identified not only in terms of their initial performance but also the stability of their response (Scarascia-Mugnozza et al. 1997).

The total number of branches was lower in the case of the 'Pegaso', AF2' and 'MC' clones than for the other clones used in the trials. These differences may affect the final percentage of bark obtained, which might in turn affect the characteristics of the biomass as a fuel since bark has a higher concentration of inorganic elements and ash (Tharakan et al. 2003). According to Lehtikangas (2001), high bark content may have a negative effect on the calorific power of biomass since it generates a greater quantity of ash which could cause a sinterization problem. Additionally, high bark content (with higher nutrient

content than wood) could also lead to loss of nutrients in the soil after wood exportation and therefore loss of fertility. This factor should be assessed when considering the necessity and intensity of treatments such as fertilization.

The differences in the distribution of the biomass as a result of the variations in tree structure, i.e., thickness of main stem, number of branches or angle of branches in relation to main stem, may also affect the logistics of harvesting in that the machinery required for this task will depend on one or other of these factors (Kauter et al. 2003).

In general, for the whole set of conditions tested, the performance of the *P. tricho-carpa* × *P. deltoides* (TxD) hybrids included in the trials was poorer than that of the *P. deltoides* × *P. nigra* (D × N) hybrids. This may be, as suggested in trials conducted in other Mediterranean countries (Dillen et al. 2007), because the latter are better adapted to the drier and warmer conditions of the Mediterranean climate thanks to the adaptive traits inherited from *P. nigra*; a pioneer species (De Vries 2000) with a high level of tolerance to environmental variations. In the case of the (D × T) × N hybrids, one of the clones tested falls within the group exhibiting the poorest growth and production ('Pegaso' clone), whereas the other displays a highly productive behaviour ('Monviso' clone). This implies that although there were only two clones, specific clones rather than genomic groups should be selected based on site conditions or geographic location, as evidenced in previous studies (Laureysens et al. 2004; Pellis et al. 2004).

It has been suggested that the differences observed between sites and among the clones tested at a given site, may be related to the length of the vegetative period for each clone and at each site (Stanton 2001; Yu et al. 2001). We have not detected this kind of association in our trials at the two localities in which the phenological data (not shown) were taken, SH and CS (the former being highly interactive and the latter exhibiting a very low level of interaction). At the SH trial site, the length of the vegetative period was similar for clones displaying very different growth and production responses such as 'AF2' and 'USA 49-177' whilst at the CS site, clones with vegetative periods of very different lengths displayed similar growth and production (the 'Monviso' and '2000 verde' clones, for example). This suggests that other factors related to soil and climate exert a greater influence (Canham et al. 1996).

Since environmental factors are highly significant, the variability must be taken into account when analysing clonal behaviour. The analysis performed highlights the potential usefulness of the biplot technique (GGE) in forest plantations where the rotation periods are notably shorter. It allows the differences and similarities among clones to be easily visualised along with the response of these clones in different environments. The clones which show high levels of interaction and which present either high ('2000 verde', 'Monviso') or low ('Unal', 'Pegaso' and 'USA 49-177') overall production, constitute a set of clones which are highly influenced by site conditions (Baker 1988). Their performance may be maximized at given locations, as evidenced by the 'Monviso' clone at the LT and VF sites or '2000 verde' at the SH site or conversely, their performance may be extremely poor, as occurred with the 'Unal' and 'Pegaso' clones at the LT, VF and AR sites or the 'USA 49-177' clone at the SH site.

The low level of interaction with site conditions displayed by the 'I-214 and 'MC' clones implies that none of the trial sites had a discernable influence on biomass production. This finding reflects the stable growth response frequently attributed to the 'I-214' clone, which is the main clone planted in many European countries for end use as lumber or plywood.

In relation to the sites, the greater level of interaction displayed at the LT and SH sites implies that the performance of the clones tested at these sites is either well above or well

below the average for these clones when the results from all the sites are taken into consideration. However, the lower level of interaction registered at the CS site indicates that the clone response in this environment approaches the average for all the trial sites as a whole. Sites with a high level of interaction, such as LT and SH, are especially interesting because the degree of differentiation among clones is greatest at such sites (Grossa et al. 2002).

The highest level of stability, and therefore the most uniform performance across all the sites was shown by the 'AF2', 'Guardi', 'I-214' and 'MC' clones, whereas the most unstable behaviour and therefore least predictable performance in the different environments was exhibited by the '2000 verde', 'Monviso', 'Unal', 'Pegaso' and 'USA 49-177' clones.

The clonal ranking for each of the different sites reveals a strong link between clonal behaviour and site conditions. Further research into these conditions is therefore required in order to determine the factors which act as constraints for each clone. The identification of these factors would facilitate optimal land use at local level.

Until other selection criteria for the tested clones are clearly understood, for example, capacity to withstand successive coppicing (Al Afas et al. 2008), response to plagues and diseases (Duplessis et al. 2009) or differing efficiency in their use of resources (Sixto et al. 2007b), it would seem reasonable from the point of view of cultivation to favour the use of productive and stable clones where specific information such as clone/site suitability is not available. If such information is known, improved performance may be attained by using productive clones which are capable of maximizing their potential under determined site conditions.

Therefore, in the Mediterranean area, in places where scarce data are available with regard to site conditions, the 'AF2' and 'Guardi' clones offer greater assurance of successful establishment and higher initial growth. Conversely, the growth of 'Monviso' '2000 verde' 'Unal' 'Pegaso' and 'USA 49-177' clones is highly dependent on site conditions during the establishment phase.

The SH (Shores Henares river) and LT (La Tallada) sites were identified as the most highly discriminative environments for the set of clones while CS (Cubo de la Solana) and AR (Atarfe) were identified as those where performance levels were average.

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