

CHARACTERIZATION OF THE INTERNATIONAL NETWORK FAIR 202 OF PROVENANCE AND PROGENY TRIALS OF CORK OAK ON MULTIPLE SITES FOR FURTHER USE ON FOREST SUSTAINABLE MANAGEMENT AND CONSERVATION OF GENETIC RESOURCES

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The international network of provenance and progeny trials was established in 1998 benefiting from homogeneous plant raising and standardize experimental design. The trials include 34 provenances that are being characterised by several teams. France 2008. Provenance PT 23 and PT+ES 25 had the lowest survival rate. In 2008 the mean height was 48.73 cm and the form keeps plagiotropic.

Italy- Sardinia, 2010 - The average mortality was 7%. Mean height and trunk DBH over cork were respectively 273 and 5.1 cm. MO I-2, TU II, ES 4-CR are above mean on height (302-309 cm), and the Italian provenances are under mean (237-249cm). Italy- Roccarespampani, Lazio- The mortality is about 45% and it is the only plantation site where the French provenance FR3 has a growth above mean. Portugal. Results show significant differences among populations on survival and growth. The Moroccan provenances show good adaptive characteristics in terms of growth, vigour and stem form. Italian provenances present lower height but higher survival. The overall mortality is around 26%. The highest growth is being observed in the Moroccan provenances and French ones showed lowest survival. Provenances and plantation sites are characterised for rainfall regime. The provenances Morocco MA27 and Tunisia TU33 that are from sites of high rainfall are among the best on growth at sites of considerable more xerothermic climate. If persistent at older age these results indicate that fast adaptation of cork oak populations to cope with climate changes leading to drier and warmer conditions seems possible.

Keywords: *Quercus suber* L., genetic variability, genetic improvement, selection, adaptation under climate change.

Parole chiave: *Quercus suber* L., variabilità genetica, miglioramento genetico, selezione, adattamento ai cambiamenti climatici.

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1. Introduction

The international network of provenance trials of cork oak was established on the frame the Concerted Action FAIR 202 “*European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies*” (from now designated as FAIR 202) financed by the European Commission. The trials offer a distinctive living material for research at cork oak. The trials are based on standardized methodologies. Seed lots were collected in 1996, all seedlings were raised at the same nursery in Portugal and sent to the different countries for plantation. The experimental design is randomized

complete block (RCB) with 2 repetitions of 2 plants per provenance (Varela, 2003). The international network of trials allows evaluating provenances for stability of growth over diverse environments

The results will provide information on genetic variability to use on delineation of breeding zones for seed supply, genetic improvement and conservation of genetic resources to cope with climate change, enhancement of resistance upon pests and diseases and new economic demands. According to Kapeller *et al.*, 2013 if climate conditions of provenance origins and planting sites are both known it is possible to derive climate response functions and/or climate transfer functions.

Since cork oak has a large distribution area with large variation in environmental conditions it is expected that disruptive natural selection (= adaptation) has caused large differentiation among populations in traits of adaptive significance such as ability to tolerate extended periods of drought, to cope with pests and diseases and varying temperature levels throughout the year (Eriksson, 2003).

2. Materials and methods

The trials of France, Les Maures, Italy, Sardinia, Grighini, Italy, Lazio, Roccarespampani, Portugal, Mogadouro, Portugal, Monte Fava and Tunisia, Tebaba include at maximum 34 provenances (Tab. 1) that are being characterised by various teams using quantitative genetics and molecular genetics to study survival, growth (mean height, diameter at breast height DBH), morphological and physiological traits in relation with geographical origin.

Provenances were selected in the economic range of cork oak and cover an important range of environmental situations although not all the climatic extreme situations where the species naturally occurs. As summer rainfall is the critical climate factor on Mediterranean region, at Figure 1 provenances are ranked after total year rainfall (P total) and P summer-accumulated rainfall of July, August and September.

The accumulated precipitation in summer has a high variation among provenances from 11 mm to 221 mm. The dryer provenances -MA29, MA28, MA26, ES8, PT19, PT23, MA30, PT18, MA27, ES11, PT21, MA31 grow in sites that get less than 50 mm during the summer months. All Morocco provenances are within the dry group, as well as four Portuguese- PT19, PT23, PT18, PT21. The Morocco provenance MA27 rainfall regime is very extreme. All French provenances grow on sites that get more than 100 mm during the four summer months.

The Tunisian provenances grow on sites with considerable high year rainfall.

The plantation sites also have considerable climatic differences on extreme temperature, rainfall and at the index of Emberger Q2 (Tab. 2), which are important to compare adaptation in various conditions.

From the initial provenance trials established in 1997/1998, there are currently provenance trials only in France, Italy, Portugal, Spain and Tunisia (Fig. 2).

3. Results

The provenances at the trials of France-Les Maures, Italy, Sardinia-Grighine, Italy, Lazio- Roccarespampani, Portugal-Mogadouro, Portugal-Monte Fava where studied on growth and mortality. Year of observation varies among trials and age is referred to the nursery phase that took place in 1997, (Fig. 3a, 3b, 4c, 4d, 4e, 4f). The provenances of the trials Portugal-Monte Fava and Portugal-Mogadouro were also studied on water use efficiency (WUE) and at the trial Portugal-Mogadouro bud burst was also studied. The results presented from Spain and Tunisia are based on published articles.

3.1 France, Les Maures

Observation on quantitative traits in 2008 at age 11 (Fig. 3a) The trial includes 33 provenances, except PT24 (Tab. 3).

Mortality in 2008 was in low rate-11.28%. However the growth rate, assessed by mean height, is the lowest among all trials- 48.73 cm. (Fig. 3a)

Highest mortality was observed at the provenance PT23 -20.8%, (PT+ES) 25 -22.2% while the lowest mortality happened on ES8, ES7, MA31, PT19 - 5.6%. The local provenance FR1 (Var, Les Maures) had a mortality above the mean but still low for cork oak plantation.

The higher growth is on Tunisian and Portuguese provenances. All French provenances exhibit a very similar growth performance, slightly below average. The Tunisian provenances T32, T33, which come from a climate more wet and mild than Les Maures (Fig. 1), succeeded a growth above-mean.

Trees keep plagiotropic habits and this is the only trial where this behaviour occurs.

3.2 Italy

3.2.1 Grighine, Sardinia

The provenance trial of Grighini includes 27 provenances (Tab. 3) and taking into consideration the combined performance on survival and growth is the best trial so far (Fig. 3b). The site is hot and dry.

Field observation at this trial has taken place regularly since plantation. In 2010, at age 13, the trial mean mortality was the lowest among all trials-6.6% and growth assessed by mean height was 273cm which is very good for cork oak (Fig. 3b). The highest mortality took place at MA26- 14.3%, yet it is a low value for cork plantation at age 13.

In 2010 the provenances MA27, TU32, ES6, AL34, PT+ES 25, TU33, MA29, MA30, ES5, PT23, PT20, PT19 showed an above average-growth contrasting with the under-average growth of all Italian provenances IT12, IT14, IT15, IT13, IT16. It is worth noting the good growth performance of the provenances of high rainfall regime MA27, TU33 (Fig. 1) at the quite dry and hot conditions of Grighini (Tab. 2) which may be an evidence of fast adaptation to dryer conditions. The correlation heightXDBH is high $R^2 = 0.8387$

3.2.2 Roccarespampani, Lazio

A field survey was performed to assess the percentage of survival of the provenances. Results generally showed a poor resistance of the species to the local environmental conditions. Only 54.88% of the plants survived after the last measurement in 2006. This would be caused both by the unfavourable climatic regime, with long-period of summer aridity, cold winter winds, and the edaphic conditions of a portion of the trial characterized by high concentration of clay. Moreover, the unexpected occurrence of badgers seriously damaged part of the field, being a further negative impact for the plants' persistence and growth." Provenance growth was assessed by mean height in 2006, at age 9 from germination in nursery (Fig. 4c). The mean height for the same age is lower than at the

trial of Monte Fava, Portugal which is probably due to the environmental site growing conditions. This is the only plantation site where the provenance FR3 has a growth above mean, at all other trials it ranks under mean (Tab. 3).

3.3 Portugal

3.3.1 Mogadouro, Quinta da Nogueira

By 2010 at this provenance trial the mortality was 26% (Fig. 4d). On survival behaviour the French provenances had the lowest survival while Italian provenances had the higher survival. The mortality is mainly due to rats.

In 2009 provenances were assessed for growth. The mean height of the trial -241cm (Fig. 4d) is not correlated with survival. Results show that the provenances ES8, ES5, ES9 and PT 20 that are from more humid climates (Fig. 1) are on the top growing group despite the drier climate profile of the plantation site (Tab. 2). On the other hand IT13 that comes from a climate similar to the trial region is the one with the lowest growth. Provenance from wet climate FR3 and IT16 seem to have less adaptability to drier climate. Significant differences between populations were observed for survival and height. Provenances were also observed over 3 years (2011-2013) on bud burst behaviour and no significant differences ($p < 0.05$) were found

3.3.2 Monte Fava

This trial is being regularly assessed but last processed data are from 2006. More recent data are the basis of the PhD thesis of Teresa Sampaio in progress.

At Monte Fava trial in 2006 all Moroccan provenances had a growth above mean (Fig. 4e) while all French provenances show a growth under the mean (Tab. 3). The Tunisian provenance TU33, the one that grows on the highest precipitation regime (Fig. 1), shows however a growth performance slightly above average; ES9 which is a provenance of low summer drought shows a growth performance slightly under the mean at the dry summer profile of Monte Fava. If these results remain at older age fast adaptation to dryer conditions shall be considered. WUE was evaluated by $\delta^{13}C$ on 11 trees per provenance. Results revealed significant differences between provenances but no correlation between height and WUE was found. Yet it must be emphasized that the Morocco provenance MA31 showed a very good growth and a good WUE (Nunes *et al.*, 2008).

3.4 Spain

Studies conducted in 2005 at the common garden experiment at Monfrague National Park, in western Spain compared thirteen Spanish provenances that includes the seven Spanish provenances selected by the EU Concerted Action FAIR 202 (Ramirez-Valiente *et al.*, 2010). Provenances were compared using total height and correlation of phenotypic traits potentially related to drought tolerance: leaf size, specific leaf area (SLA), nitrogen leaf content (Nmass) and carbon isotope discrimination (D13C).

The populations showed to be exceptionally differentiated at the locus QpZAG46 and evidence of linkage between locus QpZAG46 and genes encoding for leaf size and growth in cork oak was found. The same authors also found that *Q. suber* seedlings originating from sites characterized by more intensive droughts exhibited significantly higher survival rates than those from regions where drought is less severe i.e. not all populations are equally vulnerable to climate change (Ramirez-Valiente *et al.*, 2009).

The study foresees that under the ongoing climate change, all Iberian *Q. suber* populations are expected to incur more intensive drought periods, but Northern populations poorly adapted to dry conditions under a drier future climate will likely have lower survival.

3.5 Tunisia

On a study conducted by Khouja *et al.*, 2010 at the trial of Tebaba, Nefza, it is described that by 2005/ 2006 the trees began to show signs of decline. The study could identify that attacks were mainly due to the endophytes fungi *Biscogniauxia mediterranea* and *Discula quercina*, some new at the Tunisian cork oak pest scenario. 28 % of the trees at the trial had the crowns totally or half-dead (560 in a total of 2340 at the moment of the field observations).

The study revealed variability among provenances in terms of susceptibility to the pathogen fungi. The rate of the attacks was uneven among provenances ranging from 27% to 63 %. The Tunisian provenance Mekna (TU32) was the most sensitive and the Spanish provenance Moréna (ES7) was the less affected. (Khouja *et al.*, 2010).

On the study by Ennajeh *et al.*, 2013 at the same trial it was found that the survival of provenances felt significantly in 2011, close to half from 2004 to 2011 (Fig. 4f). The provenance PT23 (Santiago de Cacem) had the highest mortality after the pathogen fungi attack but the provenances IT13 and IT15 (Sassari, Cagliari) had low mortality. Growth evaluated by height varied from 124.6 to 101.9 cm being the trial's mean 114.66 cm (Fig. 4f).

The Tunisian provenance TU33 shows a good growth at age 14 (2011) and the susceptibility to the pathogen attack was considerable high (44.9%) although lower than for TU32 which mortality reached 52.2%. Provenance growth behaviour is also compared among trials assessed by mean height (cm) and compared to the mean height of the trial (Tab. 3).

The comparison highlights the persistently above-mean behaviour of the provenances PT20, PT23, MA27, MA28, MA29, MA30, TU32, TU33 and AL34.

4. Discussion

Guidelines for seed supply and selection of seed stands or other forest reproductive material FRM sources are still premature having into consideration that cork oak has a life span over 150 years.

Mortality varies considerable among plantation sites which lead to consider being mainly due to environmental factors than genetic.

The provenances PT20, PT23, MA27, MA28, MA29, MA30, TU32, TU33, AL34 show high stability on GenotypeXEnvironment Interaction GEI. If the good growth and GEI stability of the provenances MA27 and TU33 to plantation sites of dryer profile go on at older age the migration to dryer and warmer conditions predicted for the Mediterranean region (IPCC, 2007) seems possible. If the plagiotropic growth habits at the French trial of Les Maures continue into a later age it means it is probably due to environmental factors that must be studied in depth since it is a very negative phenotypic behavior for the exploitation of commercial cork and silvo-pastoralism.

The results on bud burst need further and more detailed observation not only because of the juvenile stage of the trees but mainly because the sub-continuous growth of cork oak leads to several events of bud burst during the year (Varela and Valdivieso, 2013). It is desirable to set standard phenologic phases to be used in further observation in all trials. Phenotypic plasticity encompasses all types of environmentally induced changes (e.g. morphological, physiological, behavioural, phenological). Detailed assessment of bud burst may be important to detect phenotypic plasticity. The high susceptibility of the Tunisian provenance Mekna TU32 to alien pathogen at the Tunisian trial of Tebaba (Khouja *et al.*, 2010) raises the importance of genetic variability and co-evolution as the key mechanisms for sustainable resistance to pests and diseases. The information on among-population genetic variability coming from the trials is essential to selection and management of

populations for dynamic conservation of genetic resources (Varela and Eriksson, 1995).

5. Perspectives

Trends of behaviour suggest that some provenances from humid climates are capable to adapt to more xerothermic climates sometimes with good growth. It is a critical result for adaptation to climate change.

Among-provenance variation in traits like growth, survival, resistance to diseases is high in some trials but insignificant at the French trial of Les Maures which may be due to the very low growth that yet does not allow differences to show. Epigenetic modification should be considered in coming studies. Harmonised methodologies for assessment of cork quality shall be put in place before the first stripping to enhance comparability of results among trials and provenances. The cork from the first stripping is unsuitable to assess quality. To speed the evaluation of cork quality it should be considered to use cork samples from the cumulative growth in the first 4 years after the first stripping (in cycles of 9-12 years) as it represents more than 60% of the whole cork production cycle (Oliveira and Costa, 2012).

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Table 1. Provenance codes, established according the arrival of seed lots for plant-growing in Portugal, at the nursery Viveiro de Santo Isidro.

COUNTRY	CODE	REGION of PROVENANCE	FOREST	NEAREST LOCALITY
France	FR1	Var	Les Maures	Bormes Les Mimosas
	FR2	Pyrenées Orientales	Le Rimbaut	Collioure
	FR3	Landes	Soustons	Soustons
	FR4	Corse	Sartene	Sartene
Spain	ES5	Montes de Toledo	Cañamero	Cañamero
	ES6	Sierra Morena Oriental	Fuencaliente	Fuencaliente
	ES7	Sierra Morena Occidental	El Carbajo	Jerez De Los Caballeros
	ES8	Parque de los Alcornocales	La Almoraima	Castellar de la Frontera
	ES9	Cataluña Litoral	Santa Coloma de Farnes	Santa Coloma de Farnes
	ES10	Sierra de Guadarrama	El Pardo	Madrid
	ES11	Alpujarras	Haza de Lino	Haza de Lino

(Table 1. Continued)

Italy	IT12	Lazio	Sughereta	Tuscania
	IT13	Puglia	Lucci-Santa Teresa	Brindisi
	IT14	Sicilia	Zotte	Catania
	IT15	Sardegna	Nuraghe Arcu de Mesu	Cagliari
	IT16	Sardegna	Puttu Addes de Subra	Sassari
Portugal	PT17	Vale do Tejo e Sado	Sociedade Agrícola Igreja Velha	Chamusca
	PT18	Vale do Tejo e Sado	Herdade da Palma	Alcácer do Sal
	PT19	Vale do Tejo e Sado	Quinta da Serra	Azeitão
	PT20	Vale do Tejo e Sado	Herdade de Vale Covo	Ponte de Sôr
	PT21	Sudoeste		S. Brás de Alportel
	PT22	Alentejo e Beira Baixa	Herdade do Paço de Camões	Azaruja
	PT23	Sudoeste	Monte Branco	Santiago do Cacem
	PT24	Trás-os-Montes e Beira Interior	Casa de Meneres	Romeo
Portugal+ Spain	PT+ES 25	Alentejo e Beira Baixa +Sierra de S. Pedro Note: the stand is crossed by the border of the two countries	Vale de Mouro + la Tojera	Besteiros + Albuquerque
Morocco	MA26	Rif Atlantique I.1	Boussafi	Larache
	MA27	Rif Occidental I.2	Aïn Rami	Chefchaouen
	MA28	Maâmora III.1	Canton A,B	Kenitra
	MA29	Maâmora III.1	Aïn Johra	Allal Bahraoui
	MA30	Plateau Central III.2	Oulmes	Oulmès
	MA31	Rif Oriental	Bab Azhar	Taza
Tunisia	TU32	Mekna	Tabarka	Aïn Sobh
	TU33	Fernana	Fernana	Aïn el Baya
Algeria	AL34	Guerbès		Guerbès

Table 2. Characterization of provenance trials sites on climate: t- mean temperature for the coldest month; T mean temperature for the hottest month; P total and P summer precipitation (mm); Q2- index of Emberger.

TRIAL	t (°C)	T (°C)	P total (mm)	P summer (mm)	Q2
France, Les Maures	1.6	29	975	181	123,2
Italy, Sardinia, Grighni	4.7	32.4	672	28	83.1
Italy, Lazio, Roccarespampani	2.4	31	868.7	108.7	104.7
Portugal, Mogadouro	0.7	28.6	554.7	60.3	69
Portugal, Monte Fava	4.3	31.3	556.6	42.7	70.8
Tunisia, Tebaba	11.1	25.6	948	65	224

Table 3. Provenance growth behaviour at the various trials assessed by mean height (cm) compared to the mean height of the trial: A-above the mean height of the trial; M – at the mean height of the trial; U – under the mean height of the trial; X-provenance not represented in the trial. For each trial it is referred the age from nursery and the year of observation. Provenances PT20, PT23, MA27, MA28, MA29, MA30, TU 32, TU33, AL34 had an above-mean growth performance at least in 4 trials. MA28 is above-mean at the 3 trials where it is represented. MA29 is the only provenance with performance on height above-mean at all of the 6 trial.

FAIR 202 provenance code		Les Maures, France age 11, 2008	Grighini, Sardinia age 13, 2010	Rocca-Respampani, Italy age 9, 2006	Mogadouro Portugal age 12, 2009	Monte Fava, Portugal age 9, 2006	Tebaba, Tunisia age 14, 2011
FR1	Var	U	X	X	A	U	X
FR2	Pyrenees Orientales	U	A	U	U	U	X
FR3	Landes	U	U	A	U	U	X
FR4	Corse	U	X	X	X	U	X
ES5	Montes_Toledo	U	A	U	A	U	A
ES6	S ^a Morena Oriental	U	A	A	U	U	A
ES7	S ^a Morena Occidental	U	U	A	A	U	U
ES8	Parque_Alcornocales	U	U	A	A	U	A
ES9	Cataluña Litoral	U	U	U	A	U	U
ES10	S ^a Guadarrama	U	M	U	M	U	A
ES11	Alpujarras	U	X	U	U	U	A
IT12	Lazio	M	U	A	U	A	U
IT13	Puglia	M	U	U	U	U	A
IT14	Sicilia	M	U	A	U	A	M
IT15	Sardegna	M	U	U	U	U	U
IT16	Sardegna	M	U	A	U	A	M
PT17	Chamusca	A	X	A	U	A	U
PT18	Herdade Palma	A	U	U	U	U	X
PT19	Q ^a Serra, Azaeitão	A	M	U	U	A	X
PT20	H Vale Côvo, Ponte Sôr	A	A	A	A	U	U
PT21	S B Alportel	A	A	U	U	U	A
PT22	H Paço Camões, Azaruja	A	A	U	U	A	U
PT23	S. Cacem, Monte Branco	A	A	A	U	A	U
PT24	Casa Meneres, Mirandela	X	X	X	A	U	X
PT+ES25	Alentejo_Beira Baixa+Sierra de S. Pedro	U	A	A	U	U	A
MA26	Rif Atlantique I.1	M	M	A	U	A	U
MA27	Rif Occidental I.2	A	A	A	A	A	U
MA28	Maâmora III.1	A	X	X	A	A	X
MA29	Maâmora III.1	A	A	A	A	A	A
MA30	Plateau Central III.2	A	A	A	A	A	U
MA31	Rif Oriental	M	X	U	A	A	A
TU32	Mekna, Aïn Sobh	A	A	A	U	U	A
TU33	Fernana, Aïn el Baya	A	A	U	U	A	A
AL34	Guerbés	U	A	U	A	A	A

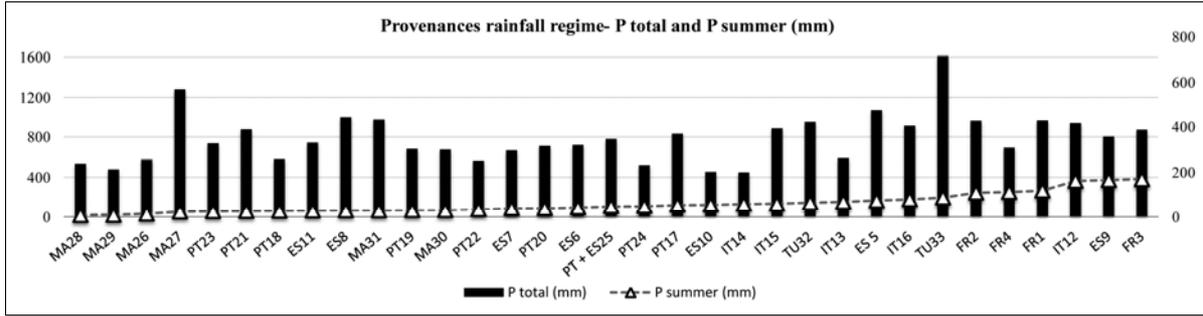


Figure 1. Climatic characterization of provenances based on precipitation: total for the year (P total) and accumulated summer in July, August, and September, P summer. Provenances have a high variability, specially on summer precipitation which is a critical factor for adaptation in Mediterranean region.

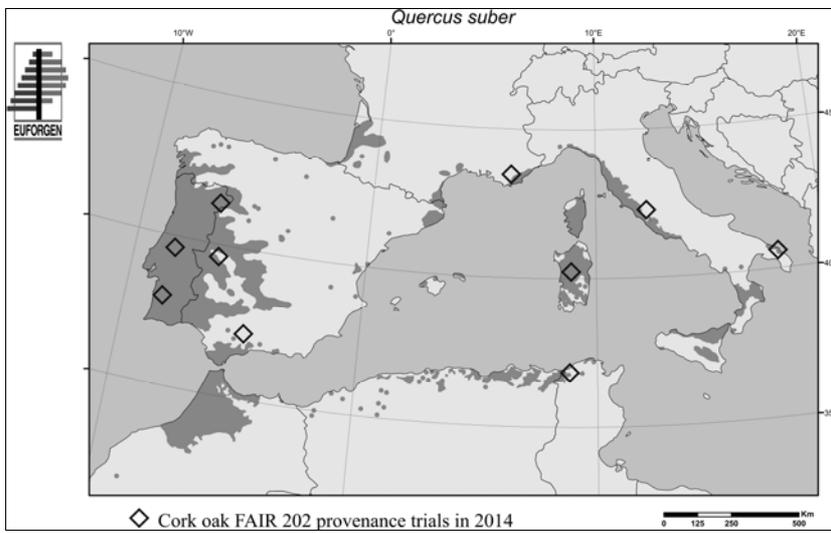


Figure 2. Cork oak FAIR 202 international network of provenances: localization of provenance trials on the EUFORGEN *Quercus suber* distribution map.

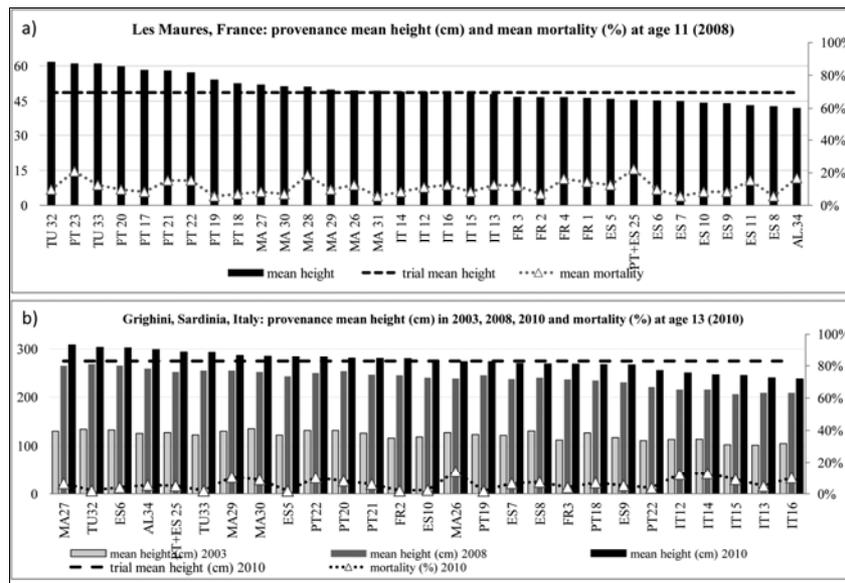


Figure 3. Provenance assessment by height and mortality on the trials of a) Les Maures, France, b) Grighini, Sardinia.

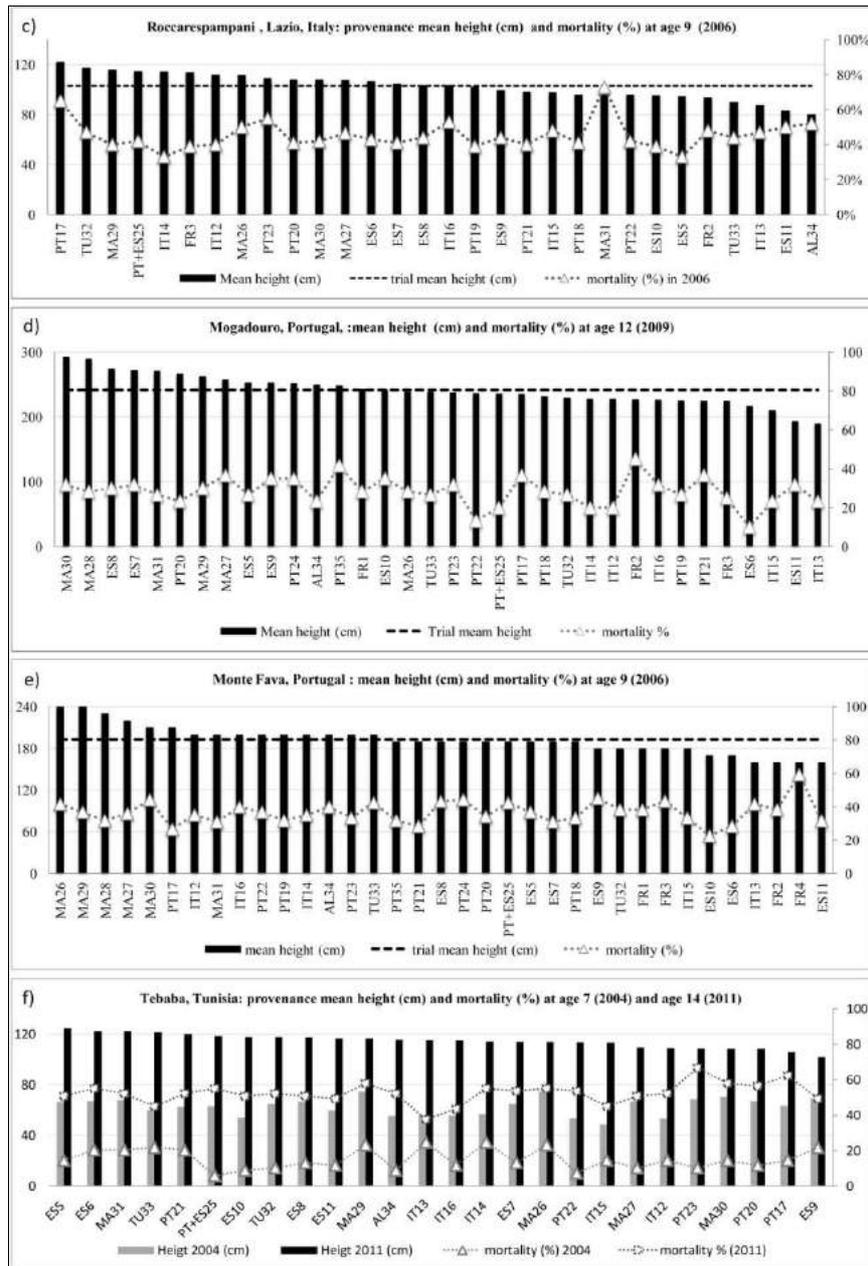


Figure 4. Provenance assessment by height and mortality on the trials of Italy, c) Roccarespanpani, Lazio, Italy, d) Mogadouro, Portugal, e) Monte Fava, Portugal, f) Tebaba Tunisia.

RIASSUNTO

La rete internazionale FAIR 202: caratterizzazione di provenienze e progenie di quercia da sughero per l'ulteriore utilizzo in sistemi di gestione forestale sostenibile e per la conservazione delle risorse genetiche

La rete internazionale di parcelle sperimentali è stata avviata nel 1998 a partire da materiale di propagazione omogeneo e con un condiviso disegno sperimentale. Le prove di campo comprendono 34 provenienze che sono state caratterizzate con l'uso di tecniche di genetica quantitativa e molecolare per studiare sopravvivenza, accrescimento, aspetti morfologici e fisiologici in relazione all'origine geografica.

Francia, 2008. La provenienza PT23 e PT+ES 25 ebbero la più bassa percentuale di sopravvivenza. Nel 2008 l'altezza media era di 49 cm e l'habitus vegetativo risultava plagiotropico.

Italia – Sardegna, 2010. La mortalità media era del 7%. L'altezza media e il diametro del fusto “a petto d'uomo” erano nell'ordine pari a 273 e 5.1 cm. MO I-2, TU II, ES 4-CR fornivano valori di altezza superiori alla media, mentre le provenienze italiane si collocavano tutte al di sotto della media.

Portogallo. I risultati mostrano differenze significative tra le popolazioni per sopravvivenza, accrescimento e capacità di adattamento (fenologia ed efficienza nell'uso dell'acqua). Le provenienze marocchine hanno mostrato buone capacità di adattamento in termini di

accrescimento, vigore e regolarità del fusto, mentre quelle italiane evidenziavano accrescimenti ridotti ma alta capacità di sopravvivenza. La mortalità generale era prossima al 26%. Il maggiore accrescimento era osservato nelle provenienze marocchine mentre quelle francesi hanno fornito la più basse percentuali di sopravvivenza.

Questi risultati dovrebbero essere tenuti da conto quando l'origine del materiale di propagazione è presa in esame in vista di estesi progetti di piantumazione dove risulta importante la capacità di adattamento, la qualità del sughero, la sensibilità a malattie e parassiti animali e per la conservazione di risorse genetiche. Le prove di progenie hanno fornito informazioni chiave per i programmi di miglioramento genetico tesi ad incrementare aspetti di importanza economica quali la qualità del sughero ovvero per approcciarsi ai problemi di consanguineità.

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