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in

Mutke S. (ed.), Piqué M. (ed.), Calama R. (ed.).
Mediterranean stone pine for agroforestry

Zaragoza : CIHEAM / FAO / INIA / IRTA / CESEFOR / CTFC
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 105

2013
pages 81-88

Characterization of *Pinus pinea* L. and *P. halepensis* Mill. provenances from Spain and Tunisia related to their rootstock use

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Abstract. Experiences carried out on the last years for exploring the possibilities of stone pine as an orchard crop have rarely been focused in the study of rootstocks and their effect on phenology and cone production. Thus, on the framework of PCI project, rootstock behaviour trials of different *P. pinea* and *P. halepensis* provenances have been planned, including an early characterization under controlled conditions, complemented on the following years with a field trial network where these plant materials will be grafted and established on different edaphic and climatic conditions in Spain and Tunisia. In 2010, seedlings of 8 Spanish and Tunisian Stone pine ('Remel', 'Mhibes', 'Aiguafreda', 'Caldes de Malavella') and Aleppo pine ('Kef', 'Thibar', 'Sallent', 'Palau-Sator') provenances were submitted to 3 water stress levels and two substrate typologies in a split-split-plot experimental design carried out at IRTA's station Torre Marimon (Caldes de Montbui, Spain). Data recorded included growth and biomass, physiologic parameters (RWC, CT, $\delta^{13}C$) and ontogenical information. Higher intra-specific variability and a more clear response to the different treatments have been observed in *P. halepensis*. Tunisian *P. halepensis* provenances showed higher biomass allocation in the more stressful conditions (particularly 'Thibar'), but displayed significant reductions on allocated biomass in sandy substrate (particularly 'Kef'); they also showed lower RWC values and a faster ontogeny (vegetative phase change).

Keywords. Aleppo pine – Stone pine – Provenance trial – Water stress – Substrate.

Caractérisation de différentes provenances de* Pinus pinea *et* P. halepensis *d'Espagne et de Tunisie par rapport à leur emploi comme porte-greffes

Résumé. *Les expériences réalisées ces dernières années explorant les possibilités du pin pignon comme culture fruitière ont rarement été orientées à l'étude des porte-greffes et de leur influence sur la phénologie et la production de cônes. Ainsi, dans le cadre d'un projet PCI, des essais de caractérisation comme porte-greffes de différentes provenances de* P. pinea *et* P. halepensis *ont été planifiés, comprenant une évaluation précoce en conditions contrôlées, qui sera complémentée ultérieurement par un réseau d'essais de terrain où ces matériels végétaux seront greffés et établis sous différentes conditions édapho-climatiques en Espagne et en Tunisie. En 2010, des plants de 8 provenances d'Espagne et de Tunisie de Pin pignon ('Remel', 'Mhibes', 'Aiguafreda', 'Caldes de Malavella') et de Pin d'Alep ('Kef', 'Thibar', 'Sallent', 'Palau-Sator') furent soumis à trois niveaux de stress hydrique et à deux typologies de substrats dans un dispositif expérimental en split-split-plot conduit à l'IRTA Torre Marimon (Caldes de Montbui, Espagne). Les données collectées incluent croissance et biomasse, paramètres physiologiques (RWC, CT,* $\delta^{13}C$*) et information concernant la différenciation ontologique. Une plus grande variabilité intra-spécifique et une plus claire réponse aux différents traitements ont été observées chez* P. halepensis*. Les provenances tunisiennes de* P. halepensis *(surtout 'Thibar') montrèrent une plus grande fixation de biomasse dans les conditions les plus stressantes, tandis que la biomasse fixée en substrat sableux fut significativement réduite (surtout pour 'Kef') ; elles montrèrent également des valeurs plus faibles de RWC et un changement plus rapide de phase végétative.*

Mots-clés. *Pin d'Alep – Pin pignon – Essai de provenance – Stress hydrique – Substrat.*

I – Introduction

Experiences carried out for exploring the possibilities of stone pine as an orchard crop have been mainly focused on the evaluation of their productive potential, in elucidating the relevance of environmental factors and in the selection of the best productive genotypes (Catalán Bachiller 1990; Mutke Regneri *et al.,* 2003; Mutke *et al.,* 2005a; Mutke *et al.*, 2005b; Mutke *et al.*, 2007). However, there is very little information (Climent *et al.*, 1997) concerning which is the suitable plant material to use as rootstock in each environment and how it affects the phenology and fruitfulness in grafted plantations.

On the framework of a PCI project, behaviour trials of different *P. pinea* and *P. halepensis* provenances under controlled conditions have been undertaken to make an early characterisation for its use as rootstocks. The main objective of the trail carried out at Torre Marimon IRTA's station has been to study the behaviour of different *P. pinea* and *P. halepensis* provenances against water stress. On the following years, this nursery characterization will be complemented with a field trial network on different edaphic and climatic conditions in Spain and Tunisia.

II – Material and methods

1. Plant material and experimental design

For the conduct of this experience a total of 8 provenances have been chosen in different ecological environments of Tunisia and Spain, trying to cover a wide range of ecological conditions (Fig. 1, Tables 1 to 3). Four of them were Aleppo pine provenances ('Kef', 'Thibar', 'Sallent' and 'Palau-Sator') and the other four stone pines ('Remel', 'Mhibes', 'Aiguafreda' and 'Caldes de Malavella'). 'Palau-Sator' and 'Caldes de Malavella' are coastal provenances, whereas 'Sallent' and 'Aiguafreda' are more continental; Tunisian stone pine provenances have a sub-humid climate, whereas Aleppo pine provenances come from semi-arid regions.

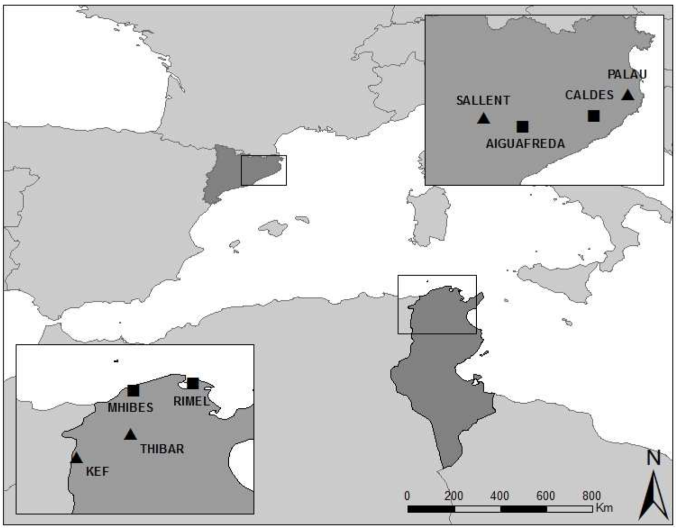

Fig. 1. Location of the *Pinus pinea* (■) and *P. halepensis* (▲) provenances.

Table 1. Geographical origin of provenances used in the trial

Provenance	Species	Origin	Long	Lat
Palau-Sator	*P. halepensis*	Spain	3º 6‘	41º 58'
Sallent	*P. halepensis*	Spain	1º 56'	41º 49'
Kef	*P. halepensis*	Tunisia	8°23'	36°12'
Thibar	*P. halepensis*	Tunisia	9° 7'	36°31'
Caldes Malavella	*P. pinea*	Spain	2º 49'	41º 50'
Aiguafreda	*P. pinea*	Spain	2º 15'	41º 46'
Mhibes	*P. pinea*	Tunisia	9° 9'	37° 7'
Rimel	*P. pinea*	Tunisia	9°58'	37°13'

Table 2. Ecological characterization of Spanish provenances used in the trial

Provenance	Altitude	Provenance region	Climate subtype	Soil†	Texture	pH
Palau-Sator	50-100	High Catalonia	Subnemoral Mediterranean	LVh	Loam	8.2
Sallent	400-500	Inner Catalonia	Nemoral subesteparian	CMc	Clay loam	8.5
Caldes de Malavella	120-135	Coastal Catalonia	Nemoromediterranean submediterranean	CMd/u	Sand loam	6
Aiguafreda	465-630	Inner Catalonia	Nemoral subesteparian	CMd	Loam	8.3

†LVh: Haplic Luvisol; CMc: Calcaric Cambisol; CMd/u: Dystric/Humic Cambisol; CMd: Dystric Cambisol.

Table 3. Climatic characterization of Tunisian provenances used in the trial

Provenance	Location	P	T	t_m	T_M	Bioclimate
Kef	North-West Tunisia	446	16.3	3.3	34	Semi-arid Mediterranean, temperate winter
Thibar	North-West Tunisia	612	17.9	5.7	35.3	Semi-arid Mediterranean, mild winter
Mhibes	North Tunisia	829	17.4	-	-	Sub-humide Mediterranean, mild-warm winter
Rimel	North Tunisia	610	18.1	7.6	31.1	Sub-humide Mediterranean, warm winter

P: annual rainfall; T: mean annual temperature, t_m: average of minimum temperatures of coldest month; T_M: average of minimum temperatures of warmest month.

Seeds were sown in April 2009 into Forest Pot-200 air-slit forest trays. In November 2009 seedlings were transplanted into Coneplast C-20R containers (2.5 l) containing two sorts of mixture: half of the total plants produced were transplanted into the Substrate 1 (S1), constituted by peat and vermiculite (2:1); the other half were transplanted into Substrate 2 (S2), constituted by peat, vermiculite and sand (1:1:1). Plants were maintained in a forest nursery until spring 2010; in June 2010 plants entered into greenhouse and the application of the different irrigation regimes started in July 2010. This experience lasted until November 2010.

Three water stress levels were applied, corresponding to minimum soil water contents of 20%, 10% and 5%, (cm^3 water/cm^3 substrate). Water supply was always of 333 ml/plant, but the frequency of application varied between treatments (Table 4). The substrate water content was monitored with a set of TDR probes (time-domain reflectometry). This information was useful to modify the frequency of irrigation in the different periods of the experiment. The experimental design was a split-split-plot with 3 replications and 20 plants per experimental unit (total size 2520 plants), with irrigation regime, substrate and provenance as factors.

Table 4. Irrigation treatments scheduling

Irrigation regime	7 Jul – 29 Jul	30 Jul – 1 Sep	2 Sep – 14 Nov	SWC†
R1	333 ml / 2 days	333 ml / 3 days	333 ml / 3 days	20%
R2	333 ml / 3 days	333 ml / 5 days	333 ml / 6 days	10%
R3	333 ml / 4 days	333 ml / 7 days	333 ml / 9 days	5%

†Minimum Soil Water Content, prior to re-watering.

2. Measurements

Data were recorded at three different moments, at the beginning, middle and end of the trial (final June, final August and November). In each sampling date, plant height, plant diameter and biomass determinations of each portion, below and aboveground (root, stem, needles) were registered. These data were used to define some relative variables (allocated biomass of each fraction and root/shoot ratio). Moreover, some physiologic parameters, relative water content (RWC) and cuticular transpiration (CT) were recorded. Water use efficiency (WUE_i) was estimated from the isotopic carbon content ($\delta^{13}C$) analysis of some control plants not subjected to water stress. Finally, ontogenetic information (adult needle proportion and winter buds setting) was also noted down.

III – Results

1. Differences between species

At species level, very significant differences were observed in final biomass, total allocated biomass and allocated aerial biomass, with higher values in *P. pinea* in all the irrigation regimes and for the two types of substrate; root/shoot ratio at the end of the trial and root/shoot increase from the middle to the end of the trial were significantly higher in *P. halepensis*, in all the irrigation regimes and for the two types of substrate (Table 5).

Table 5. ANOVA for different biomass variables

Species	B_f (g)	R/S_f	ΔR/S	ΔB (g)	ΔB_root (g)	ΔB_aer (g)
	***	***	***	***	N.S.	***
P. halepensis	12.78 b	0.58 a	0.17 a	8.33 b	2.63	5.71 b
P. pinea	19.21 a	0.39 b	0.09 b	10.43 a	2.51	7.92 a

B_f: Biomass at the end of the trial; R/S_f: root/shoot at the end of the trial; ΔR/S: root/shoot increase; ΔB: Total allocated biomass; ΔB_root: Total allocated root biomass; ΔB_aer: Total allocated aerial biomass. Duncan (α=0.05); N.S.: non-significant; ***: significant P<0.001%.

Concerning the physiological variables, very significant differences between species were observed in cumulated cuticular transpiration rate and relative water content, with higher values in *P. pinea* in all the irrigation regimes and for the two types of substrate; the proportion of secondary neddles was always significantly higher in *P. halepensis*; no significant differences at species level were found for the isotopic carbon content (Table 6).

Table 6. ANOVA for different physiological variables

Species	CCT_m (slope)	RWC_m (%)	Sn (% dry weight)	$\delta^{13}C$ (‰)
	***	***	***	N.S.
P. halepensis	0.044 b	88.09 b	10.25 a	27.71
P. pinea	0.049 a	91.86 a	0.87 b	27.93

CCT_m: Cumulated cuticular transpiration rate in the midle of the trial; RWC_m: needles relative water content in the midle of the trial; Sn: Proportion of secondary needles at the end of the trial; $\delta^{13}C$: isotopic carbón content. Duncan (α=0.05); N.S.: non-significant; ***: significant P<0.001%.

2. Differences within species

A. Pinus halepensis

Although there are no significant differences, Tunisian *P. halepensis* provenances, and particularly 'Thibar', showed a slightly higher biomass allocation in the more stressful conditions (Fig. 2a). Tunisian *P. halepensis* provenances, and particularly 'Kef', displayed significant reductions on allocated biomass with the sandy substrate; 'Palau-Sator' and 'Sallent' are much less affected by the type of substrate (Fig. 2b).

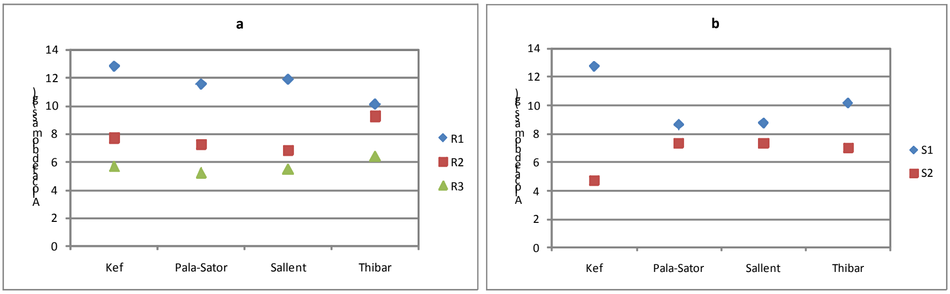

Fig. 2. Allocated biomass of *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

'Palau-Sator' shows the highest root to shoot ratios and 'Kef' the lowest ones (Fig. 3, Table 7); however, 'Sallent' and 'Thibar' undergo a more clear response to the water stress treatments, with a significant increase of shoot to root ratios in the more stressful conditions (Fig. 3).

Tunisian Aleppo pine provenances had the lowest relative water content values, in both favourable and stressful scenarios (Table 7). Concerning isotopic carbon content, we found highly significant differences within *P. halepensis* provenances: 'Palau-Sator' and 'Sallent' are placed on the highest and the lowest WUE_i position, respectively; Tunisian provenances ranged in intermediate position (Table 7).

Tunisian Aleppo pine provenances (and particularly 'Kef') showed a faster ontogeny (vegetative phase change) represented by a higher proportion of secondary needles (Table 7) and a higher frequency of budset (data not shown). Budset and occurrence of secondary needles was always lesser in sandy substrate (Fig. 4).

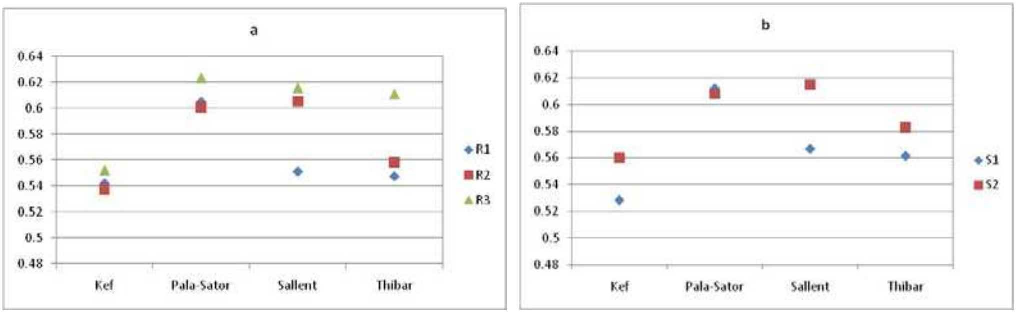

Fig. 3. Root to shoot ratio of *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

Table 7. ANOVA between *P. halepensis* provenances for different variables

Provenance	Root/shoot	RWC_m (%)	Sn (% dry weight)	$\delta^{13}C$ (‰)
	*	***	***	***
Palau-Sator	0.61 a	90.2 a	3.0 c	-27.24 a
Sallent	0.59 ab	89.2 a	1.7 c	-28.17 c
Thibar	0.57 bc	86.6 b	14.9 b	-27.48 b
Kef	0.54 c	86.4 b	20.8 a	-27.95 c

RWC_m: needles relative water content in the midle of the trial; Sn: Proportion of secondary needles at the end of the trial; $\delta^{13}C$: isotopic carbón content. Duncan (α=0.05); N.S.: non-significant; *: significant P<0.05%; ***: significant P<0.001%.

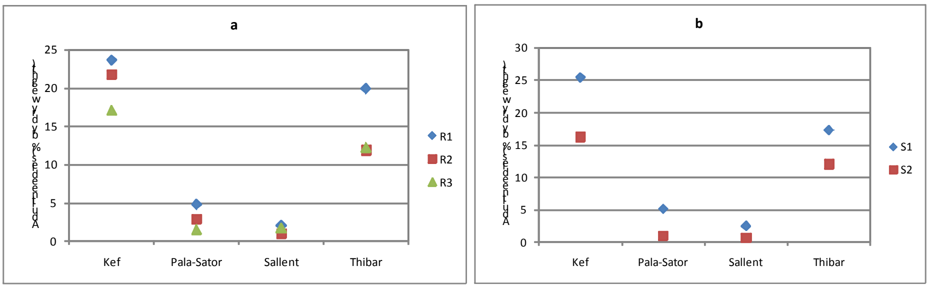

Fig. 4. Percentage (by dry weight) of secondary needles in *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

B. Pinus pinea

Concerning water stress treatments, there are no significant differences in allocated biomass between *P. pinea* provenances (Fig. 5a). On the other hand, the type of substrate entails significant differences between provenances: sandy substrate has a negative effect in Tunisian *P. pinea* provenance, whereas 'Caldes de Malavella' seems to be indifferent to the type of substrate (Fig. 5b). Tunisian *P. pinea* has the higher root to shoot ratio, even in the more stressful conditions (Table 8).

There are no significant differences between *P. pinea* provenances in relative water content and isotopic carbon content (Table 8). Occurrence of secondary needles was very low in *P. pinea* provenances (Table 8); we also observed lesser budset in Tunisian stone pines (data not shown).

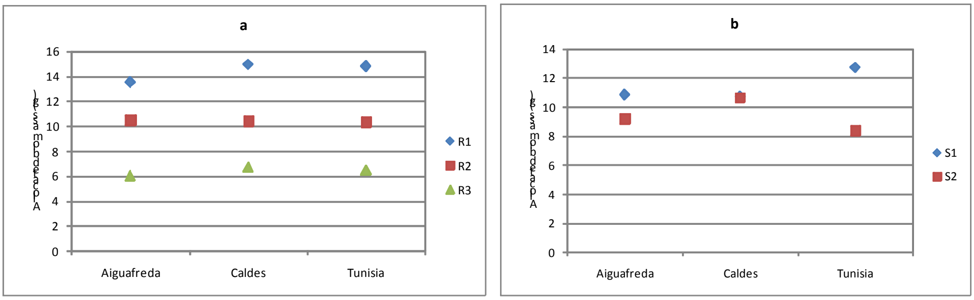

Fig. 5. Allocated biomass of *P. pinea* provenances: response to water stress levels (a) and to the type of substrate (b).

Table 8. ANOVA between *P. pinea* provenances for different variables

Provenance	Root/shoot	Sn (% dry weight)	$\delta^{13}C$
	***	N.S.	N.S.
Tunisia	0.41[†] a	1.01[†]	-27.89[††]
Aiguafreda	0.37 b	0.88	-27.94
Caldes de Malavella	0.40 a	0.72	-27.95

Sn: Proportion of secondary needles at the end of the trial; $\delta^{13}C$: isotopic carbón content. Duncan (α=0,05); N.S.: non-significant; ***: significant P<0,001%

[†]'Tunisia' provenance is constituted by 'Rimel' and 'Mhibes' seedlings.
[††]The value of isotopic carbon content corresponds only to Rimel provenance.

IV – Conclusions

In general, higher intra-specific variability and a more clear response to the water stress treatments and to the different substrates have been observed in *P. halepensis*, whereas *P. pinea* shows low variability in most of the variables analyzed. Stone and Aleppo pine Tunisian provenances are much more affected by the type of substrate than the Spanish ones.

The aim of this study was to make an early characterization of different plant materials in front of water stress. The next step will be to graft these plant materials and to establish a network of experimental plots in different ecological conditions, in Spain and in Tunisia, in order to study its field behaviour and the influence of different rootstocks in growth, phenology and cone production of grafted materials.

Acknowledgments

Project supported by the Program of Scientific Cooperation and Interuniversity Research (PCI), funded by the Spanish Agency for International Development Cooperation (AECID).

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