Relationships between seed weight, germination potential and biochemical reserves of Maritime Pine in Morocco: Elements for Tree Seedlings Improvement

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Journal: Forest ecology and management

Abstract

Selection of quality seeds in breeding programs can significantly improve seedling productivity. Germination and biochemical analyses on seeds from ten natural populations of maritime pine (*Pinus pinaster Ait*.) in Morocco reveals significant differences among populations in seed weight, germination characters and protein content in both dry seeds and megagametophytes. During germination, the mobilization of protein content in megagametophyte is significantly different among populations than sugar content. A strong positive correlation between the germination capacity and the protein content in both dry seeds and megagametophytes indicates that the best populations in term of germination capacity may also be the richest in protein content. The present study finds that seed weight is not a good indicator for quality seed selection, nor is it recommended to increase the degree of germinability.

Our results suggest that the pine population in southern Morocco might have adapted to drought conditions as it is characterized by heavy seed weight and lower speed of protein content mobilization in megagametophyte compared to northern populations growing in temperate climate.

Keywords: maritime pine, seed weight, germination capacity, biochemical reserves.

Introduction

A taxon of the atlantico-mediterranean region, the maritime pine (*Pinus pinaster Ait*.) is the most abundant tree species in Morocco and represents therefore an important natural resource in terms of both economic benefit and land protection. Geographically, it is found in the High and Middle Atlas Mountains, the Rif Mountain and the Mediterranean coastal region (Destremau, 1974; Quezel, 1980). The discontinuity of the mountainous landscape combined with human activities, have led to the fragmentation and isolation of this species' population. To date, the potential of the genetic diversity of Moroccan maritime pine populations has not been exploited in programs of breeding, planting or reforestation of the species. As a result this species continues to degrade under increased anthropogenic pressures such as exploitation of forest resources, deforestation, overgrazing and fires (FAO 2002). Furthermore, harsh climatic conditions characterized by warming and droughts during the last couple of decades, led to an overall ecological degradation affecting the natural regeneration and decreasing the species' areal coverage (M'Hirit 1999, Zine El Abidine 2003). The safeguard and perennity of this tree species and its genetic diversity may be preserved by programs of conservation and improvement of the quality of its phytogenetic resources. In this context, propagation by sowing remains one of the principal methods of safeguard of genetic diversity (e.g.; Pita et al. 1998) and selection of traits related to seed germination would be benefic for breeding programs.

Seed quality has a great impact on the quality of seedlings. For example, it has been shown that early leaf emergence is important to the successful establishment of seedling and represents a significant determinant in total dry mass of 8-week-old eastern white pines (El-Kassaby et al. 1992, Parker et al. 2006) while it is suggested that seed germination parameters can influence seedling characteristics (Parker et el., 2006).

For the last 20 years, the seedlings production technology advanced greatly and so did the seed quality, however not for the Moroccan maritime pine.

It is generally accepted that the food reserve in seed is a key step for obtaining satisfactory germination rates in plants (e.g.; Bray 1995). The reserve of carbohydrates and protein are important energy sources for the developing embryo during germination and early growth (Bewley and Black 1994, Bonfil 1998). Consequently, knowledge of the control and the optimization of the germination rate and mobilization of biochemical reserves of seeds have a considerable importance in the selection of quality seeds and seedlings' production technological advance.

In this study we *i)* evaluate the variation in seeds' germination potential and biochemical reserves (proteins and sugars) of 10 natural populations of maritime pine from Morocco, *ii)* assess the mobilization of biochemical reserves during germination, and *iii)* examine the relationships between seeds' characteristics, germination potential, biochemical reserves and geographical indicators.

Material and Methods

Plant material

We use samples of seeds from 10 populations distributed over three distinct biogeographical regions over Morocco (the Rif, the Middle and the High Atlas). The principal geographic and climatic attributes of all populations are provided in Table 1. For each population, and following ecological gradients represented by altitude and slope exposure, 16 to 35 trees were sampled at a rate of 10 to 20 cones per tree except for the *Tamrabta* population, destroyed by wild fires, for which a batch of seeds was provided to us by the seeds' centre of the National Forestry Commission of the region of Azrou (Morocco). The cones were exposed to the sun

for one month and seeds from each cone of each tree and population, were then extracted manually and appropriately stored at 4°C for proper conservation.

Seed weight

For each tree of each population, we selected 12 individual seeds and measured their weight using a precision balance. Per population average weights were computed and used for further analysis.

Seed germination

The seeds were subjected to germination tests following ISTA (International Seed Testing Association) (1999) procedures. Seeds were soaked in bleach solution with a concentration of 10 % during 10 min and then washed with distilled water. The germination tests were carried out in Petri dishes (10 cm of diameter) on top of two Whatman paper disks moistened with distilled water. The seeds were incubated at 25 C° during the day and 17 C° at night, with 12 h/12 h light/darkness regime under an irradiance of 22.44 Watt/m^2. The relative humidity in the incubator was maintained at 80 % (±2 %) throughout the entire test period. Eight batches of 50 seeds per Petri dish were gathered from each population, for a total of 400 seeds per population. Following Côme (1970), a seed was considered to have germinated when its radical became visible with a length of 5-10 mm. Seeds showing radicle emergence were counted every two days and removed from the Petri dishes during the 48 days germination period. The following seed germination characteristics were assessed: *1)* the mean germination time, T_m measured in days representing the number of days needed to obtain 50 % of germinated seeds; T_m is an indicator of the speed of germination of each population, and *2)* the germination capacity, G_c measured in percent and representing the number of germinated seeds recorded after 48 days of seed incubation (Côme, 1970).

Biochemical analysis

Eight batches of 25 seeds per population were selected at different germination stages and a standard biochemical analysis was performed on three different samples from each batch.

The biochemical analysis of seeds and megagametophytes of the 10 populations was then carried out by measuring the quantities of total soluble proteins and free sugars (carbohydrates reserves) contents. The evolution of the energy mobilization in the megagametophytes was assessed through quantification of total proteins and free sugars contents at each stage of the germination at intervals of 0, 5, 10 and 15 days from the beginning of the germination, with stage zero indicating that the seeds were soaked in water only for 12 hours.

Extraction and analysis of total soluble protein content

For the extraction of proteins, we took 100 mg of fresh material (FM) separately from the seed and the megagametophyte and crushed it in cold mortar containing 2 ml of buffer extract (0.1M Tris-maleate (pH= 6.5) and 0.5 % Triton-X-100) following El Hadrami et al. (1995). The homogenate was then centrifuged at 4 °C for 3 minutes at 7000 x *g* and the pellet was soaked twice in 1 ml of buffer extract. The supernatant was used as a crude protein extract. The total protein was measured by spectrophotometer at 595 nm following Bradford (1976). Bovine Serum Albumin (BSA) standard was used to determine the total protein concentration.

Extraction and analysis of free soluble sugars content

The extraction method of free sugars content follows the procedure described in Booji et al. (1992). Free sugars were extracted from 100 mg FM of seed and megagametophyte in 2 ml of 80 % aq. ethanol. After heating the extract for 30 minutes at 100 °C, the homogenate was centrifuged at 4 °C for 3 minutes at 7000 x *g*. Analysis of the free soluble sugars was performed using the phenol-sulfurique method (Dubois et al., 1956). The free soluble sugars

were measured by a spectrophotometer at a wavelength of 485 nm using glucose solution as a standard basis.

Statistical analysis

All statistical analyses are carried out using SAS® statistical package, version 9.2 (SAS 2009). Normal distribution and homogeneity of variance are assumed and validated using standard graphical methods and tests with the UNIVARIATE procedure. Germination data were subjected to the *arcsin* transformation to satisfy these assumptions. Data of germination and biochemical characteristics are analyzed using the one way analysis of variance ANOVA following the linear model:

$$Y_{ij} = \mu + p_i + \varepsilon_{ij}$$

Where Y_{ij} is an observation made on the j^{th} individual sampled from the i^{th} population, μ is the overall mean, p_i is the random effect of the i^{th} population, and ε_{ij} is the random residual effect associated with the j^{th} individual sampled from the i^{th} population. Population means are calculated for each germination and biochemical characteristics. In addition, means are compared using *Tukey* test for each series of ANOVA analysis. Correlation between pairs of germination and biochemical characters is evaluated using Pearson's correlation coefficient.

Results

Variation in Seed germination and biochemical characters

Differences among populations for seed weight, germination capacity (G_c), germination time (T_m) and total protein content (T_p) are all statistically significant at the 95 % confidence level as shown by the one-way ANOVA tests. However, for the free sugar content and at different

stages of the seed (dry seed or megagametophyte after germination), these differences are not statistically significant within this confidence interval (Table 2).

Seed weight characteristics

The highest mean values for seed weight are found in the populations of Madisouka (Mad), Tadiwine (Tad) and Jbel-Bouhachem (Jb) from the Rif, Talaghine (Talgh) from the Middle Atlas and Sidi-Meskour from the High Atlas. The population of Punta Céres (Pc) has the lowest mean values of seed weight (0.044 ± 0.0072 g) (Table 3).

Seed germination characters

The mean values for the germination capacity (G_c) and germination time (T_m) under controlled conditions, 25°C during 12 h of light and 17 °C during 12 h of darkness, for all populations are presented in Table 3. Most populations from the Rif (Pc, Kr, Tad, Jb) and Middle Atlas (Talgh, Tamj) reveal a high germination capacity ranging between 88 and 95 %. In contrast, the population from Sidi-Meskour (Sm) from the High Atlas presents a low germination capacity of only 62 %.

The mean germination time for all populations varies between 10.4 days for Jb in the central Rif and 17.2 days for Pc in the occidental Rif with a median value of 14.8 days (Table 3).

Biochemical characters

Total protein contents in both dry seed and megagametophyte are presented in Figure 1. Punta Céres (Pc), an Occidental Rif population, shows the highest level of total protein content per fresh matter (FM) in both dry seed (19.31 mg/g FM) and megagametophyte (12.25 mg/g FM). The lowest total protein content in dry seed (14.75 mg/g) and megagametophyte (7.2 mg/g) is found in Sm and Adl (14.14 and 7.8 mg/g FM, respectively).

Our analysis shows that only two populations: Talgh (Middle Atlas) and Mad (Rif Oriental) present high free sugar content in dry seed, more than 90 mg/g, while all other populations'

free sugar content is below 83 mg/g. Differences in free sugar content in dry seed between these two populations and all others are statistically significant at the 95% level. Comparison among populations using the *Tukey* test shows that free sugar content in megagametophyte (Figure 2) is more homogeneous among populations than the protein content.

Mobilization of biochemical reserves during germination

Analysis of total protein content mobilisation in megagametophytes as function of the germination time shows that protein content increases from the imbibition stage until the fifth day of germination for all populations (Figure 3). After that it monotonically decreases for all populations except for Kr which exhibits a slight increase between the fifth and tenth of germination. After 15 days of germination, relatively large and statistically significant variations in total protein content are found among populations (Table 2) with the highest mean value (13.81 mg/g FM) of protein content at Tamr (Middle Atlas) and the lowest (8.84 mg/g FM) in Jb, a Rif population (Figure 3).

The free sugar content mobilization in megagametophyte as a function of germination time shows a 3-phases pattern (Figure 4). During the first phase, between imbibition and the 5^{th} day of germination, the free sugar content remained relatively stable for Tamr, Tamj, Jb and Pc and decreased slightly for other populations. Between the 5^{th} and 10^{th} day of germination however, the free sugar content decreases rapidly with about the same mobilization speed for Kr, Mad, Adl and Jb populations, and a slightly higher speed for Talgh, Tamr and Tamj. The populations in Tad and Pc present the slowest decrease in free sugar mobilization during this phase. In the third phase, between the 10^{th} and 15^{th} day of germination, the slope of the decrease in free sugar mobilization in megagametophytes is within the same range for most populations except for Tamj which had a relatively slower mobilization (Figure 4). The ANOVA analysis does not reveal any statistically significant variation in the free sugar content in megagametophytes after 15 days of germination (Table 2).

Correlations

Our analysis shows high positive correlations between the germination capacity and the protein content in dry seed (r= 0.64) and megagametophyte (r= 0.91) (Figure 5). Similarly, the mean germination time is correlated with total protein content in megagametophyte after 15 days of germination (r= 0.53) (Figure 6).

Table 4 shows that the seed weight is negatively correlated to the germination capacity and the total protein content in both dry seeds and dry megagametophytes. It is strongly correlated with the total protein content in dry seed (r= -0.68) and only moderately correlated to the total protein content in dry megagametophyte (r= -0.46) and in megagametophyte after 15 days of germination (r= -0.34). The seed weight is also moderately correlated to the mean germination time (r= -0.41). Except for the correlation with the total protein content in dry seed, none of the others correlations is statistically significant at 95% (Table 4). Interestingly, the seed weight presents the lowest correlation with the germination capacity (r= -0.17) explaining only about 3% of its variance, a rather counterintuitive result.

Among populations, the germination capacity and the total protein content in dry seeds as well as in megagametophytes vary with geographical locations. The highest germination capacity and total protein content in dry seed and megagametophyte are observed in the northern populations. On the hand, the seed weight and the protein content in megagametophyte after 15 days of germination seem to decrease for north-western populations whereas the mean germination time increases for eastern populations.

Discussion

Seed, germination and biochemical characters

Our results confirm the high level variability of seed weight, germination characters and protein content in seeds and megagametophytes among populations of maritime pine in Morocco. We find that most of the variance in seed weight is due to the population genetic differences, a finding consistent with previous results, on Moroccan maritime pine populations, reporting high variance not only in seed weight but also in seed length, width, depth and wings' length and width (Wahid et al., 2006 and references therein). This population-induced variance in seed weight is also in line with results from similar studies on seeds from other conifers (ST. Clair and Adams 1993, Sorensen and Campbell 1993). The pronounced inter-population variation in seed weight may be explained by the genetic variability that is present in this species and confirmed to exist in other Mediterranean provenances of maritime pine (Resch 1974, Destremau et al. 1976). In general, environmental influences during the development of seeds combined with genetic variability can result in variations in seed dimensions (Sorensen and Campbell 1993). Indeed, important variations in climatic conditions of the natural distribution of maritime pine in Morocco (Table 1), led Boudy (1950) to suggest that the habitat (provenance) effect leads to pronounced differences among populations. This expression of the provenance on seed weight variance could add up to the population genetic differences (Sorensen and Campbell 1993). Thus, for seed source trials, and better gene conservation, it is desirable to capture as much as possible of the potentially valuable genetic variation among populations.

The reduced environmental variation associated with controlled incubation conditions of 12 h/12 h light/darkness at specific respective temperatures, enables us to detect the genetic variation in the degree of germinability among populations. This genetic variation is known to exist in conifers such as Douglas-fir (El-Kassaby et al., 1992), red cedar (Zine El Abidine et al. 1999) and Austrian pine (Mataruga et al. 2010). Some of this variation may be of genetic origin but much of it is due to environmental stresses associated with local conditions under

which the seed matured. Discrimination of seed germination characters may be particularly important for seedling survivorship and selection of the best material. For example, for seeds landing on soil surface under stressful environmental conditions, a difference in germination time and germination potential may be crucial for a successful establishment (Hillet 1972). Moreover, in dense stands, seedlings established first may have an important competitive advantage (Chaisurisri et al. 1992). This variability in seed germination among maritime pine populations can be very useful in advanced breeding and deployment programs to select the best seeds in term of germinate potential for reforestation and plantation stock.

This study also reveals a high variation in protein content in dry seed and megagametophyte among populations. This variation can be explained either by genetic control as suggested by Limami et al. (2002) or by the influence of environmental conditions on mother trees (Bénétrix and Autran 2001). Indeed, previous studies, Durzan and Chalupa (1968), noted that soluble protein levels in seeds and female gametophytes of jack pine were affected by differences in ecological conditions and habitats. These results provide insight into genetic improvement in the seed food reserves quality (in particular protein content) of locally adapted populations.

Biochemical mobilization during germination stages

During the period between the seed's imbibition and the five-day germination, the total soluble protein content increases for all populations. In the strict sense, the time between seed imbibition and radicle emergence is the period of germination during which seeds require water uptake and swelling followed by biochemical activity and synthesis of new metabolites from mRNAs' translation formed during seeds maturation and which become functional only during the early stages of germination (Bewley and black 1994, Lea and Ireland 1999). In fact, increases in the total protein content in the early stages of germination are attributable to

the synthesis of abundant soluble proteins necessary to the mobilization of the gametophytes' food reserves during germination development (see also: Durzan and Chalupa 1968). This result is consistent with that of Gifford (1991) who found increases in DNA, RNA and protein quantities of lodgepole pine's megagametophytes and embryos after 2 to 5 days of seeds germination. These increases are accompanied by the synthesis of a subset of proteins specific for a post germination period of seed development. Then after 5 days of germination, the total soluble protein content decreases for all populations, revealing the mobilization of protein reserve in megagametophytes necessary to support the high energy needs for germination. In contrast during the first five days of germination the free sugar content remains basically unchanged in the megagametophytes. After that, utilization of the free sugar content occurs between the fifth and fifteenth day of germination with varying speeds for different populations. This suggests a high mobilization of sugar content in megagametophytes during this period to initiate and develop the growth of the embryo during germination.

The present study shows a high variance in total protein content after 15 days of germination with different mobilization patterns among populations. However, the free sugar content in the megagametophytes after 15 days of germination is relatively homogeneously mobilized among all populations. These results suggest that for maritime pine seeds, the expression of the genetic variability is higher for total protein than for free sugar content in megagametophytes and call for the argument that the total protein content is much more influenced by the genetic and environmental effects (see also: Mataruga et al. 2007). Similarly, Durzan and Chalupa (1968), expected that climate at seed source will affect the degree to which some biochemical food reserves metabolic conversions proceed and often extend their influence well into the seedling development.

Correlations

The present study shows a high positive correlation between germination capacity and total protein content in both dry seeds and megagametophytes, indicating that the selection of the best population in germination capacity may also have a high richness in seed protein content. In addition, moderate and statistically significant correlations are revealed between the mean germination time and the total protein content after 15 days of germination suggesting that germination speed requirements depend, at least partially, upon content and functional machinery of seed protein. Our results are consistent with those reported by Bonfil (1998) showing a high germination potential associated with the richness in seed protein content. This finding suggests that for the Moroccan maritime pine, beside genetic and environmental effects, the relative heterogeneity in germinability behaviour is dependent on the seed total protein content and its mobilization.

Seed size is a widely accepted measure of seed quality because large seeds have high seedling survival, establishment and growth rates (Spurr 1944). However, although a marginal correlation between seed weight and mean germination time is shown, similar to previous studies we find a weak correlation between seed weight and germination capacity. For example, Chaisurisri et al. (1992) found that seed size of *Sitka spruce* has little effect on seed germination. Thus seed weight selection is not recommended to increase the degree of germinability, and sowing unsorted seeds is indeed good for increasing genetic diversity. In contrast, seed weight is negatively correlated with the total protein content in dry seeds, dry megagametophytes and germinate megagametophytes (after 15 days of germination), indicating that seed weight is not a good indicator for quality seed with high protein content.

Our results show that seed germination capacity and protein content in dry seeds and megagametophytes are highest for the north-eastern populations (i.e. *Pc, Kr, Jb, Tad* from the Rif and *Tamj* from the High atlas) and lowest for the south-western populations (i.e. *Sm* from High Atlas). Indeed, populations in northern regions grow in a humid climatic zone under

both Atlantic and Mediterranean influences whereas the southern populations belong to the semi-arid to sub-humid climate under the Atlantic and Saharan influences and in which moisture availability presents an important north-south gradient. Climate conditions appear to be determinant to the seed's germination capacity and protein content in dry seed and megagametophyte of the maritime pine in Morocco. In a study examining the germination of ponderosa pine seed from 225 sites, Weber and Sorensen (1992), suggested that some of the geographic variations in speed of seed germination were related to the severity of summer droughts with germination speeds greater in locations with drought-limited growing seasons. Durzan and Chalupa (1968) noted that most biochemical contents in different parts of the seed were remarkably associated with climate factors. It has been shown in some coniferous species that seeds from drought-tolerant sources have harder and thicker seed coats than seeds from drought-sensitive sources (Côme, 1970). Our results suggest that the population of maritime pine in south Morocco (e.g.; *Sm* in the High Atlas) might have adapted to drought conditions as it is characterized by heavy seed weight and lower speed of protein mobilization in megagametophyte compared to northern populations (*PC*) growing in temperate climate temperature and humidity regimes.

Conclusion

Analysis of seeds from different natural maritime pine populations in Morocco reveals significant differences in weight, degree of germinability and protein content in seeds and megagametophytes with most of the variance attributed to population genetic effects. This variability appears to be related to the diversity of habitats which act to favour genotypes adapted to local climatic conditions.

This analysis shows a high correlation between the germination capacity and the protein content in both dry seeds and megagametophytes, indicating that the selection of the best population in germination capacity is also rich in protein reserves. Consistent with previous

results, we find a statistically significant correlation between the mean germination time and the protein content past 15 days of germination confirming that germination speed requirements depend, at least partially, on the content and metabolism of seed protein. We also show that the protein content is more sensitive to genetic and environment effects than the sugar content, at least during the first 5 days of germination. The negative correlation between seed weight and total protein content in dry seed, dry megagametophyte and germinate megagametophytes suggests that seed is not a good indicator for quality seed protein content.

The relationship between geographical locations, seed germination and biochemical characters in different population groupings, may form a basis for the development of bio-indicator models to predict seed quality responses to environmental stresses. However, seeds from habitats in favourable climatic conditions are likely to be of superior quality regardless of where they are sown.

Acknowledgements

We are grateful to the centre of seeds of the National Forestry Commission of Azrou for providing seed information for the maritime pine population of Tamrabta.

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Tables and Figures

Table 1: Geographic and climatic characteristics of 10 natural populations of maritime pine (*Pinus pinaster Ait*.) in Morocco. T_{max} represents the annual mean maximal temperature (^{o}C), T_{min} the annual mean minimal temperature (^{o}C) and RR the annual total precipitation (mm).

Populations	Abbreviation	Altitude (m)	Latitude	Longitude	RR	T_{max}	T_{min}
Rif Occidental							
Punta Céres	Pc	40	35°55'N	5°28' W	709	29.5	10.8
Koudiat-Erramla	Kr	400	35°28'	5°23'	824	32.5	7
Rif Central							
Jbel-Bouhacheme	Jb	1400	35°14'	5°25'	258	32.5	4
Adeldhal	Adl	1450	35°08'	5°09'	5	32.4	4
Madisouka	Mad	1360	35°11'	5°10'	976	32.4	4
Tadiwine	Tad	1600	34°56'	4°32'	976 1251	27	2
Middle Atlas							
Tamjout	Tamj	1550	33°50'	3°59'	422	32	1
Talaghine	Talgh	1840	32°27'	5°14'	362	31	-2
Tamrabta	Tamr	1650	33°36'	5°01'	763	30	-2
High Atlas							
Sidi-Meskour	Sm	1910	31°28'	6°50'	386	38	-3.2

Note: although peaks in the Rif are similar to those in the Middle Atlas, temperatures in the Rif are modulated by the effect of the Mediterranean Sea.

Table 2: Variance analysis, mean squares and F-values of seed germination and biochemical characters of maritime pine in Morocco. FM denotes fresh matter.

Characteristics	Mean squares		F	Probability
	within population	between populations		P-test
Seed characteristic				
Weight	0.00012	0.00093	7.518	0.000
Germination characteristics				
Germination capacity (%)	0.627	3.768	53.424	0.000
Mean germination time (days)	282.19	416. 82	6.010	0.000
Biochemical characteristics in dry seed				
Total protein content (mg/g FM)	46.46	89.687	4.290	0.003
Free sugar content **(**mg/g FM)	2277.17	1273.3	0.950	0.500
Biochemical characteristics in megagametophyte				
Total protein content after 12 hours imbibition (mg/g FM)	338.29	442.24	2.905	0.023
Free sugar content after 12 hours imbibition **(**mg/g FM)	183.631	50.173	0.607	0.777
Total protein content after 15 days of germination (mg/g FM)	37.492	174.191	5.162	0.009
Free sugar content after15 days of germination **(**mg/g FM)	33.469	10.107	0.336	0.824

Table 3: Mean, standard deviation and Tukey test for seed weight, germination capacity (G_c) and mean germination time (T_m) of 10 natural maritime pine populations in Morocco. Homogeneous groups, statistically significance at 95% confidence level are indicated by the same letter.

	Pc	**Kr**	**Mad**	**Adl**	**Tad**	**Jb**	**Talgh**	**Tamj**	**Tamr**	**Sm**
***Seed* Weight**										
Mean (g)	0.044	0.057	0.061	0.058	0.065	0.062	0.062	0.055	-	0.061
SD	*± 0.007*	*± 0.009*	*± 0.012*	*± 0.012*	*± 0.011*	*± 0.011*	*± 0.010*	*± 0.012*	-	*± 0.010*
Tukey	*B*	*AB*	*A*	*AB*	*A*	*A*	*A*	*AB*	-	*A*
Seed germination characters										
G_c (%)	92	89	80	76	95	93	88	95	71	62
SD	*± 0.024*	*± 0.046*	*± 0.035*	*± 0.023*	*± 0.019*	*± 0.023*	*± 0.042*	*± 0.018*	*± 0.020*	*± 0.027*
Tukey	*A*	*A*	*AB*	*AB*	*A*	*A*	*A*	*A*	*AB*	*B*
T_m (days)	17.2	15.5	14.5	15.1	15.2	10.4	14.8	13.5	13.7	16.7
SD	*± 0.416*	*± 0.51*	*± 0.516*	*± 0.700*	*± 0.99*	*± 0.45*	*± 0.600*	*± 0.420*	*± 0.728*	*± 0.486*
Tukey	*B*	*AB*	*AB*	*AB*	*AB*	*A*	*AB*	*AB*	*AB*	*B*

Table 4: Pearson coefficient of correlation between seed germination capacity, germination time, protein content in dry and germinate seed/megagametophyte of maritime pine populations in Moroccan.

	Seed weight
Germination capacity	-0.175 (*0.654*)
Mean germination time	-0.414 (*0.269*)
Protein content in dry seed	-0.684 (*0.042*)
Protein content in dry megagametophyte	-0.459 (*0.214*)
Protein content in megagametophyte after 15 days of germination	-0.344 (*0.365*)
Seed weight	1

Note: The numbers between brackets represent the P-values.

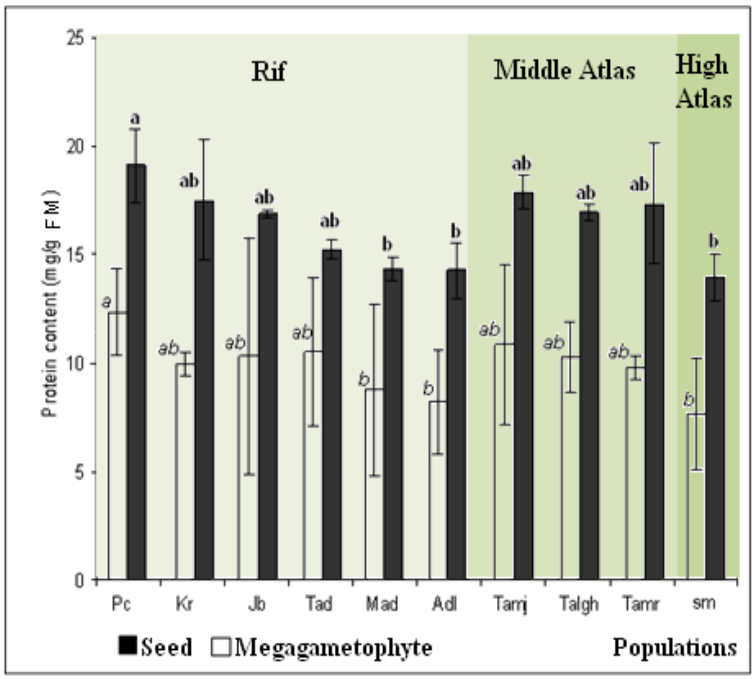

Figure 1: Protein content in dry seeds and megagametophytes of 10 natural populations of maritime pine in Morocco. Homogeneous groups statistically significant at the 95 % limit are indicated by the same letters. Color is used to delineate populations from different biogeographical regions.

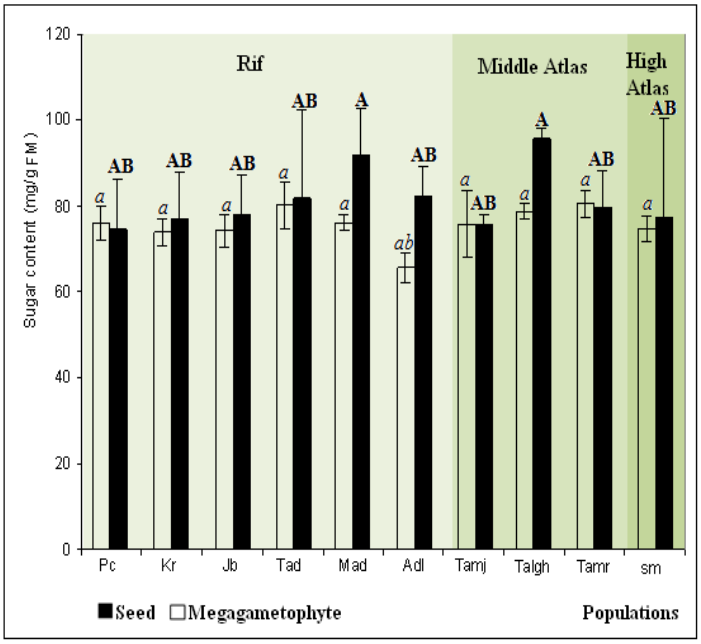

Figure 2: Free sugar content in dry seeds (dark squares) and megagametophyte (clear squares) of 10 natural populations of maritime pine in Morocco. Homogeneous groups, statistically significant at 95 % limit, are indicated by same letters. Bars represent standard deviations from the mean of each population. Color is used to delineate populations from different biogeographical regions.

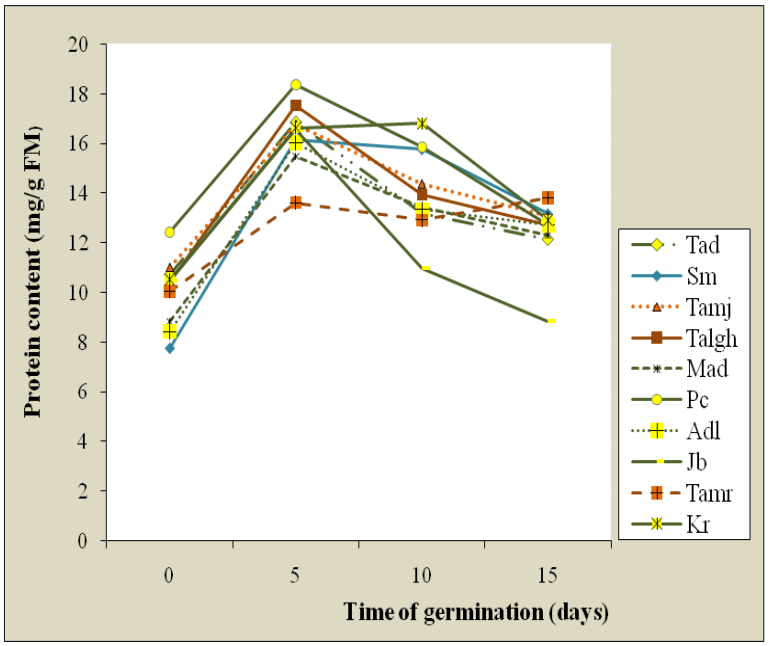

Figure 3: Mobilization of protein content in megagametophytes as a function of germination time of 10 natural maritime pine populations in Morocco.

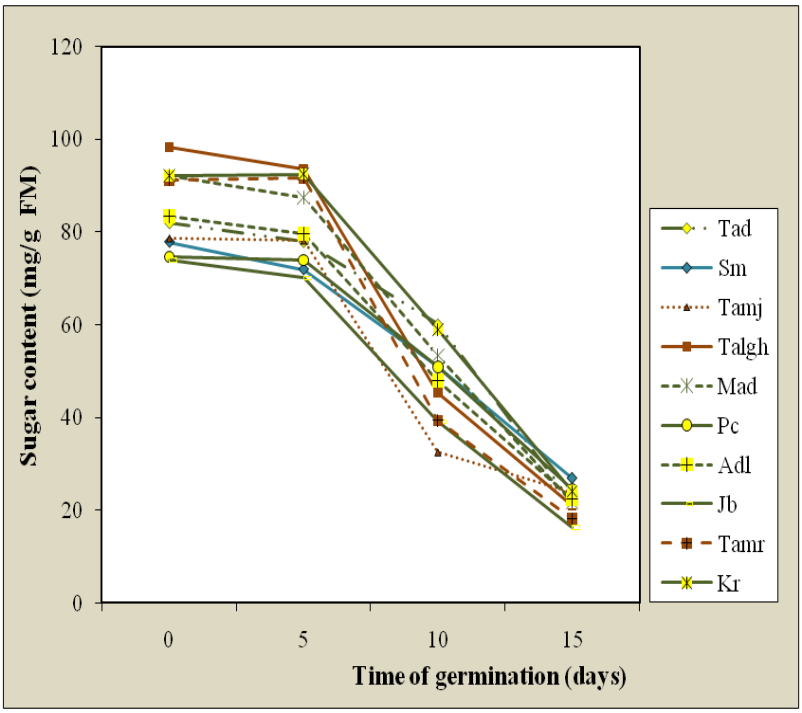

Figure 4 : Mobilization of free sugar content in megagametophytes as a function of germination time of 10 natural maritime pine populations in Morocco.

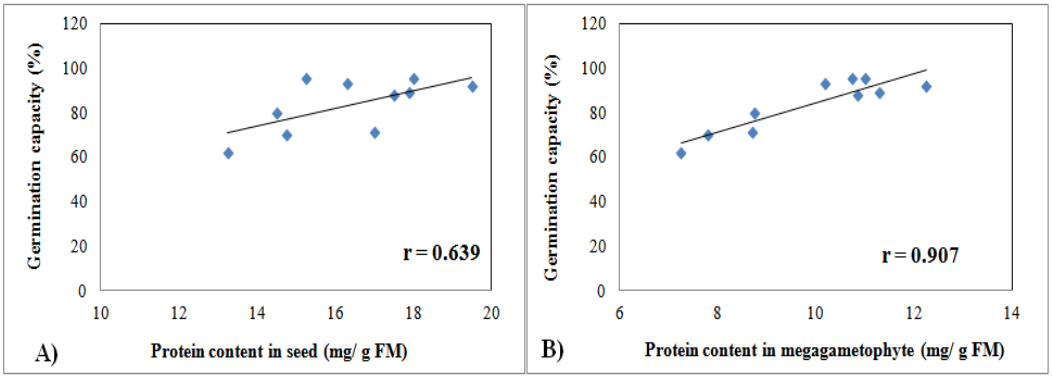

Figure 5: Correlation between germination capacity and protein content in: A) dry seed and B) dry megagametophyte. Linear trend lines with correlation coefficients (r) are also shown.

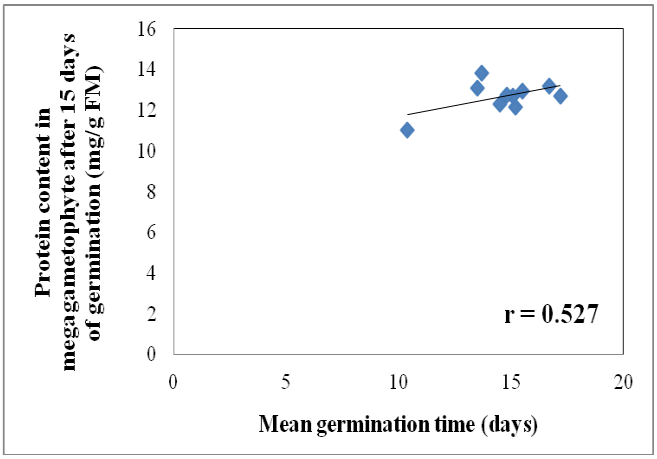

Figure 6: Correlation between mean germination time and protein content in megagametophytes after 15 days of germination. Linear trend lines with correlation coefficient (r) are also shown.