# Variation and Population Structure in Aleppo pine (*Pinus halepensis* Mill.) in Algeria

By A. Harfouche[1,2], S. Boudjada[1], W. Chettah[1], M. Allam[1], O. Belhou[1] and A. Merazga[1]

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## Abstract

In Algeria, *Pinus halepensis* covers about 850 000 ha in coastal and inland areas where populations are subjected to various selection pressures. A few studies have shown a substantial difference between population variation in phenotypes for adaptive traits and morphology suggesting geographic and ecotypic differentiation within the species. The present work, which is based on 49 open pollinated families of eight stands of Aleppo pine from the western coastal region of Algiers, is intended for getting information on within-population variation.

There were small differences between stands for most traits suggesting that little genetic differentiation between stands exists within the studied region. Parameters as size and location of stands may play a role in shaping genetic variation within stands. This variation was generally of lesser importance in small and/or coastal stands. Given the size of the studied area, it was not expected to find marked links between traits and geographic variables such as altitude or longitude; nevertheless, it was observed west-east clines of variation for 100-seed weight and wing width, parallels with an increase in annual rainfall from western to eastern parts of the zone.

As a direct implication for selection, it may not be of interest to retain for seed use purposes stands or restricted areas when prospecting at a local scale; rather, priority should be given to selecting families and/or individuals. And, at the whole population level, because of the weak level of differentiation among stands, it would be sufficient to preserve only a few of stands for ensuring efficient gene conservation.

*Key words:* Aleppo pine, stand variation within a region of provenance, seed, cone, plantlet traits.

## Introduction

Aleppo pine (*Pinus halepensis* Miller) is a member of the *halepensis* pine group which comprises also brutia pine (*Pinus brutia* Tenore) and its various forms. It is nowadays largely accepted that *Pinus halepensis* is a distinct species from its eastern vicariate *Pinus brutia* (Mirov, 1955; Mirov *et al.,* 1966; Nahal, 1962; Allegri, 1974). Studies, using morphological, physiological and/or biochemical traits and markers, were set out to refine phylogeny and taxonomy of this species complex, as to investigate genetic variation at the intra-specific level (Iconomou *et al.,* 1964; Palmberg, 1975; Bellefontaine, 1979; Pelizzo and Tocci, 1978; Schiller and Grunwald, 1987; Schiller *et. al.,* 1986; Calamassi, 1986; Fisher *et al.,* 1986; Conkle *et al.,* 1988; Schiller and Waisel, 1989; Gallis and Panetsos, 1997; Gallis *et al.,* 1998; Agúndez *et al.* 1999; Matziris, 2000). Aleppo pine is a circum-mediterranean species whose range stretches over 28 degrees of longitude and 15 degrees of latitude. However, this range is rather centred to the western Mediterranean (Mirov, 1967; Panetsos, 1981; Thanos and Skordilis, 1987). In eastern Mediterranean, populations often are scattered and relic (Nahal, 1962; Weinstein, 1989).

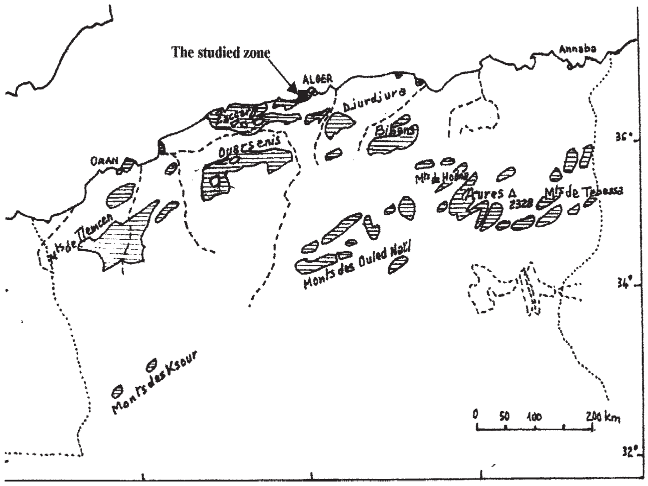

*Figure 1.* – The Aleppo pine range in Algeria.

In Algeria, *Pinus halepensis* covers about 850 000 (Boudy, 1953; Kadik, 1986) in coastal and inland areas (*Fig. 1*) where populations are subjected to various selection pressures. A few studies have shown a substantial geographic variability in phenotypes for adaptive traits and morphology (Kadik, 1986) suggesting geographic and ecotypic differentiation among populations; however, investigations in common environments are necessary to determine whether differences were inherent or environmental in nature. Moreover, data are rare, not to say non-existent, which dealt with individual and/or within population variation; therefore, needs are great for information about genetics and population structure of the species. Then, one of our main objectives with this work was to fill some gaps in this field. Investigations on morphological attributes of cones, seeds and seedlings are carried out on stands from a coastal Aleppo pine region of provenance. Morphological traits of cones and seeds can be estimated from natural stands (Dorman, 1976), in that they are likely less strongly influenced by environmental conditions than are physiological or growth related traits. Plantlet traits were assessed in nursery.

## Material and Methods

49 open pollinated families of eight stands of Aleppo pine from the western coastal region of Algiers were included in the study. This region is particularly interesting to study because of its ecological diversity and the occurrence of a typical „coastal facies“ of the species, first described by Maire (1926). The prospected zone extends over about 15 km (as the crow flies) parallel to the Mediterranean coast and over about 3 km in depth in the back country (*Fig. 2*). Geographic and ecological data as well as the number[1] of families for each stand are pre-

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1) INRF, Arboretum de Baïnem, B.P. 37, Cheraga, Alger, Algerie. Tel. 213 (0) 21 90 12 33. Fax 213 (0) 21 90 12 48.

2) Senior author to whom all correspondence must be addressed (E-mail: a_harfouche@hotmail.com

[1] These numbers varied from 1 to 14 in proportion to the size of the stands.

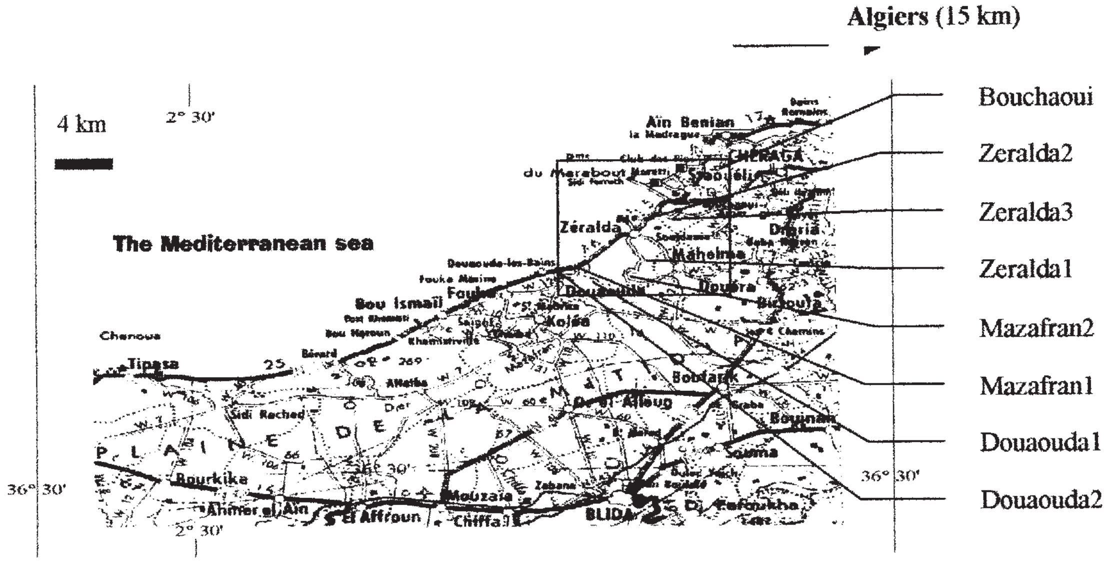

*Figure 2.* – The studied region.

*Table 1.* – Characteristics of stands and sample sizes. ***Long.** = kilometric longitude; ****Alt.** = average altitude of the collected trees.

| Stand | Number of families | | Total size of samples | | | Soil | Order of size | Long * | Alt. ** |
|---|---|---|---|---|---|---|---|---|---|
| | seed-cone | Plantlet | Cone | Seed | Plantlet | | | | |
| Douaouda subcoastal (Doua1) | 4 | 4 | 16 | 120 | 77 | Deep red silty clayey | A few hundreds of trees | 0.0 | 46 |
| Douaouda coastal (Doua2) | 1 | 1 | 4 | 30 | 20 | Deep sandy (Dune) | A few dozens of trees | 1.0 | 10 |
| Mazafran coastal (Mazafran1) | 3 | 3 | 12 | 90 | 56 | Deep red silty clayey | A few scattered trees (A dozen) | 2.5 | 17 |
| Mazafran subcoastal (Mazafran2) | 6 | 6 | 24 | 180 | 116 | Deep red silty clayey - | Thousands of trees | 3.5 | 17 |
| Zeralda subcoastal (Zeralda1) | 3 | 7 | 12 | 90 | 127 | Deep brown, clayey | Thousands of trees | 4.5 | 54 |
| Zeralda coastal (Zeralda2) | 4 | 6 | 16 | 120 | 108 | Deep sandy (Dune) | Thousands of trees | 7.5 | 9 |
| Zeralda-game reserve (Zeralda3) | 5 | 14 | 20 | 150 | 274 | Deep red silty clayey | Thousands of trees | 10.5 | 20 |
| Bouchaoui | 4 | 8 | 16 | 120 | 80 | silty clayey | Thousands of trees | 15.0 | 110 |
| *Total* | *30* | *49* | *120* | *900* | *935* | | | | |

*Table 2.* – Overall Mean, maximum and minimum for stand effect, F-tests and probabilities for stand and family within stand for each variable. WL: wing length; WW: wing width; SL: seed length; SW: seed width; ST: seed thickness; 100-SW: 100-seed weight; CL: cone length; CW: cone width; NCOT: cotyledon number; LHYP: hypocotyl length; LOCOT: longest cotyledon; SHOCOT: shortest cotyledon; COTANISO: cotyledon anisotropy = LOCOT/SHOCOT.

| Variable | Stand | | | F-test | | *Probability* | |
|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Stand | Family(stand) | *Stand* | *family(stand)* |
| **WL (mm)** | 21.43 | 20.71 | 22.55 | 0.41 | 8.33 | *0.89* | $<10^{-9}$ |
| **WW (mm)** | 7.09 | 6.78 | 7.61 | 2.08 | 5.61 | *0.09* | $10^{-9}$ |
| **WL/WW** | 3.06 | 2.80 | 3.30 | 2.25 | 5.65 | *0.07* | $10^{-9}$ |
| **SL (mm)** | 5.94 | 5.66 | 6.62 | 0.77 | 9.51 | *0.62* | $<10^{-9}$ |
| **SW (mm)** | 3.42 | 3.23 | 3.51 | 0.71 | 7.48 | *0.66* | $<10^{-9}$ |
| **ST (mm)** | 2.23 | 2.08 | 2.27 | 1.53 | 8.71 | *0.21* | $<10^{-9}$ |
| **SL/SW** | 1.74 | 1.68 | 1.93 | 1.48 | 3.87 | *0.22* | $3.10^{-6}$ |
| **SL/ST** | 2.67 | 2.56 | 2.94 | 1.32 | 5.04 | *0.28* | $18.10^{-9}$ |
| **SW/ST** | 1.54 | 1.50 | 1.58 | 0.75 | 2.53 | *0.63* | *0.001* |
| **WL/SL** | 3.62 | 3.22 | 3.96 | 1.81 | 13.51 | *0.13* | $<10^{-9}$ |
| **WW/SW** | 2.08 | 1.97 | 2.20 | 1.64 | 6.40 | *0.17* | $<10^{-9}$ |
| **W100S (mg)** | 1964.8 | 1575.2 | 2258.2 | | | | |
| **CL (mm)** | 82.92 | 73.69 | 87.38 | 1.59 | 6.32 | *0.19* | $<10^{-9}$ |
| **CW (mm)** | 50.21 | 45.10 | 56.15 | 1.43 | 5.18 | *0.24* | $11.10^{-9}$ |
| **CL/CW** | 1.65 | 1.52 | 1.76 | 1.43 | 2.06 | *0.24* | *0.009* |
| **NCOT** | 7.68 | 7.30 | 7.97 | 2.22 | 5.73 | *0.05* | $<10^{-9}$ |
| **LHYP (mm)** | 14.75 | 13.40 | 15.89 | 1.67 | 5.53 | *0.14* | $<10^{-9}$ |
| **LOCOT (mm)** | 20.65 | 19.25 | 22.10 | 0.79 | 8.42 | *0.60* | $<10^{-9}$ |
| **SHOCOT (mm)** | 20.19 | 18.41 | 21.05 | 0.87 | 8.99 | *0.53* | $<10^{-9}$ |
| **LOCOT/LHYP** | 1.45 | 1.26 | 1.62 | 2.22 | 3.20 | *0.05* | $<10^{-9}$ |
| **COTANISO** | 1.020 | 1.008 | 1.052 | 2.78 | 1.70 | *0.01* | *0.004* |

sented in *Table 1*. The distance between collected trees was 80–100 m in order to minimize collection on related individuals. Individuals retained as mother trees were not selected in order to provide representative samples.

Globally, the climate of the zone is sub-humid with, however, a gradient of annual rainfall from western (628 mm) to eastern (717 mm) boundaries. A relative variety in soils is encountered within this region, but deep sandy and red silty or clayey soils are the most widespread.

Seed attributes were assessed on a sample of 30 trees (*Table 1*) at the rate of 30 seeds and 4 cones (8, 8, 7, 7 seeds, respectively) per tree. Seeds were sown in nursery in bottomless polythene bags (9 cm diameter, 20 cm height) at the rate of 2 seeds/bag and 20 replicates per family. As soon as cotyledons spread 15[2] to 20 plantlets per family were reserved for recordings and measurements. At the age of 8 months, they were transplanted in 10-liter pots for further investigations. The traits assessed for cones, seeds and plantlets are defined in *Table 2*. The trait called "cotyledon anisotropy" expresses the situation where the cotyledons of one side of the rosette were longer (shorter) than the cotyledons of the other side. It is taken as the ratio of the longest cotyledon to the shortest one.

*Statistical analyses*

For seed traits, a 3-factor hierarchical model was used in analysis of variance:

$$Y_{ijkl} = \mu + s_i + t(s)_{ij} + c(t(s))_{ijk} + e_{ijkl} \qquad (1)$$

where µ is the grand mean, si is the random stand effect (i = 1, 2,..., 8), t(s)ij the random mother-tree within stand effect (n =

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[2] Rate of germination was poor for certain families.

1-14), c(t(s))ijk the random effect of cone within mother tree within stand (n = 4) and eijkl the random error. Variance components resulting from the analysis were used to calculate the following parameters:

$$G_d = \sigma^2_s \Big/ \sigma^2_s + \sigma^2_{t(s)} + \sigma^2_{c(t(s))} + \sigma^2_e \tag{2}$$

which gives information on stand differentiation,

$$t = \sigma^2_{t(s)} \Big/ \sigma^2_{t(s)} + \sigma^2_{c(t(s)} + \sigma^2_e \tag{3}$$

the intra-class coefficient which measures the degree of resemblance between members of a same class (Mother-tree seed lot within stand).

A one-way (stand classification) and a hierarchical two-way (stand and tree within stand classifications) analyses of variance were carried out for 100-seed weight and cone traits, respectively.

Variation in plantlet characters was analysed by means of a hierarchical two-way analysis of variance whose model was as follows:

$$Y_{ijk} = \mu + s_i + f(s)_{ij} + e_{ijk} \tag{4}$$

where Yijk is the individual (phenotypic) measure, µ the grand mean, si the random stand effect (i = 1, 2,..., 8), f(s)ij the random open-pollinated-family within stand effect (n = 1-14) and eijk the random error. The following parameters were also calculated:

$$G_d = \sigma^2_s \Big/ \sigma^2_s + \sigma^2_{f(s)} + \sigma^2_e \tag{5}$$

where $\sigma^2_s$ is the stand component of variance, $\sigma^2_{f(s)}$ is the family within stand component of variance and $\sigma^2_e$ the error variance. As for seed and cone traits, Gd measures the extent of between-stand differentiation;

$$t = \sigma^2_{f(s)} \Big/ \sigma^2_{f(s)} + \sigma^2_e \tag{6}$$

*t* is the intra-class (family) coefficient from which heritability is derived. Given the nature of the plant material (open-pollinated families) and as there is no evidence that selfing is significant in Aleppo pine, we took a coefficient of relationship of $^1/_4$. Thus, heritability was calculated as $h^2 = 4t$.

To some traits, there were small but significant effects between stands, as it will be seen later; it is decided to consider all of the stands to be parts of a same base population and derive intra-class coefficients and heritabilities from the whole sample of stands.

Univariate analyses were completed by principal component and tree clustering analyses which were run on stand means, with a view to obtain stand multivariate profiles and detect meaningful structures of variability which were not by univariate techniques. For clustering analysis, Euclidian distance was used and the aggregation algorithm was the *weighted pair-group method using arithmetic averages* (WPGMA) (SNEATH and SOKAL, 1973).

## Results

As seen from *Table 2*, there was small differences between stands for most traits, but cotyledon anisotropy (COTANISO) and, to a lesser extent, the number of cotyledons (NCOT) and the ratio of the longest cotyledon to the hypocotyl length (LOCOT/LHYP). Consequently, very low coefficients of differentiation between stands (Gd = 0-10%) were recorded suggesting that genetic differentiation was small among stands within the studied area. Conversely, within-stand (family) variation was moderately high for COTANISO and high for the other traits as attested by large F, *t* and $h^2$ values (*Table 3*). Heritabilities were strong for plantlet traits as the number of cotyledons (NCOT, $h^2$ = 0.79), the length of hypocotyl (LHYP, $h^2$ = 0.77) and the length of cotyledons (LOCOT and SHOCOT, $h^2$ = 1.12-1.18); on the other hand, heritability was low for cotyledon anisotropy ($h_2$ = 0.14). So, there was evidence for strong genetic differentiation between individual trees within stands. Cone within tree was a significant source of variation for most seed traits, but it was very small compared to tree effect. It can be regarded as an environmental source of variation related to cone size which varied within a tree. For plantlets, the (error) variance, expressed as a coefficient of variation (data non shown), was more or less important depending on the trait under consideration. It ranged from as low as 2.6% for LOCOT/LHYP to as high as 25.2% for LHYP. Coefficients of variation were on average lower in stands of Douaouda1, Douaouda2, Mazafran1 and Zeralda2 than in others. Two hypotheses could explain the weak plantlet differences in these stands: (i) the small size of the stand for Douaouda1, Douaouda2[3] and Mazafran1), (ii) the relative isolation by sea for the coastal stands (Douaouda2, Mazafran1 and Zeralda2), which can receive pollen or seeds solely from one direction.

*Table 3.* – Variance components and genetic parameters. $\sigma^2_{s:}$: Stand variance; $\sigma^2_{t(s)}$: Tree (or family) within Stand variance; $\sigma^2_{c(t(s))}$: Cone within tree within Stand variance; $\sigma^2_e$: Error variance; Gd: Geographic coefficient of differentiation.

| | | Components of Variance | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Traits** | $\sigma^2_s$ | $\sigma^2_{t(s)}$ | $\sigma^2_{c(t(s))}$ | $\sigma^2_e$ | **Gd** | *t* | $h^2$ |
| **Seed and Cone** | **WL** | 0.000 | 3.066 | 0.692 | 7.315 | 0.00 | 0.28 | |
| | **WW** | 0.068 | 0.192 | 0.013 | 1.144 | 0.05 | 0.14 | |
| | **WL/WW** | 0.015 | 0.036 | 0.007 | 0.177 | 0.07 | 0.16 | |
| | **SL** | 0.000 | 0.163 | 0.043 | 0.252 | 0.00 | 0.35 | |
| | **SW** | 0.000 | 0.041 | 0.016 | 0.067 | 0.00 | 0.33 | |
| | **ST** | 0.002 | 0.014 | 0.003 | 0.029 | 0.04 | 0.30 | |
| | **SL/SW** | 0.001 | 0.004 | 0.003 | 0.024 | 0.03 | 0.13 | |
| | **SL/ST** | 0.002 | 0.018 | 0.007 | 0.085 | 0.02 | 0.16 | |
| | **SW/ST** | 0.000 | 0.001 | 0.001 | 0.021 | 0.00 | 0.06 | |
| | **WL/SL** | 0.013 | 0.056 | 0.000 | 0.151 | 0.06 | 0.27 | |
| | **WW/SW** | 0.003 | 0.013 | 0.000 | 0.108 | 0.02 | 0.11 | |
| | **CL** | 11.665 | 61.180 | | 45.556 | 0.09 | 0.57 | |
| | **CW** | 3.482 | 23.981 | | 22.704 | 0.07 | 0.51 | |
| | **CL/CW** | 0.001 | 0.007 | | 0.026 | 0.04 | 0.21 | |
| **Plantlet** | **NCOT** | 0.032 | 0.127 | | 0.510 | 0.05 | | 0.79 |
| | **LHYP** | 0.367 | 2.594 | | 10.890 | 0.03 | | 0.77 |
| | **LOCOT** | 0.000 | 5.385 | | 13.815 | 0.00 | | 1.12 |
| | **SHOCOT** | 0.000 | 5.646 | | 13.440 | 0.00 | | 1.18 |
| | **LOCOT/LHYP** | 0.003 | 0.012 | | 0.102 | 0.02 | | 0.42 |
| | **COTANISO** | 0.00013 | 0.00018 | | 0.00486 | 0.03 | | 0.14 |
| | **Average** | | | | | 0.02 | | 0.73 |

NOTE: Heritabilities greater than 1.00 can be recorded in some circumstances such as assortative matings (homogamy), G x E correlation and/or G x E interaction. In the case of traits such as cotyledon size, non genetic maternal effects connected to mother-tree (nutritious) environmental factors could increase the resemblance within progenies, hence overestimating heritability.

*Table 4* gives coefficients of correlation between seed, cone and plantlet attributes, on the one hand, and kilometric longitude and altitude, on the other hand. Wing width and 100-seed weight were significantly correlated to kilometric longitude at both stand (*r* = 0.92** and *r* = 0.72*, respectively) and family

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[3] A sampling error could also be invoked, one single tree being collected from this stand. However, relative to its size (A few dozens of trees), this effect would not be necessarily more higher for this stand than for others. In addition, genes carried by the tested plantlets are assumed to be representative of the gene pool in the stand under random mating hypothesis.

levels ($r = 0.58^{**}$ and $r = 0.43^{*}$, respectively); these traits were getting higher from western part to eastern part of the zone (*Fig. 3* and *4*). Cotyledon anisotropy was significantly correlated to altitude ($r = 0.42^{*}$).

*Table 4.* – Correlation between cone, seed and plantlet traits and geographic variables (Kilometric longitude, altitude) at the stand (Df = 6) and the tree (and family) within stand (Df = 28) levels.

| | Stand level | | Tree (family) level | |
|---|---|---|---|---|
| Trait | Longitude | Altitude | Longitude | Altitude |
| WL | -0.11 | -0.04 | -0.28 | 0.10 |
| WW | **0.58**** | 0.27 | **0.92**** | 0.55 |
| SL | 0.18 | 0.01 | 0.01 | -0.13 |
| SW | 0.31 | -0.07 | 0.67 | -0.08 |
| ST | -0.21 | 0.35 | 0.54 | -0.27 |
| W100S | **0.43*** | 0.11 | **0.72*** | 0.22 |
| CL | 0.14 | 0.21 | 0.23 | 0.18 |
| CW | -0.10 | 0.07 | -0.21 | 0.39 |
| NCOT | 0.26 | -0.03 | 0.39 | -0.11 |
| LHYP | 0.30 | 0.29 | 0.55 | 0.65 |
| LOCOT | 0.30 | 0.20 | 0.42 | 0.31 |
| SHOCOT | 0.34 | 0.12 | 0.58 | 0.25 |
| COTANISO | -0.21 | **0.42*** | -0.12 | 0.30 |

In bold are the statistically significant coefficients (* p<0.05; ** p<0.01).

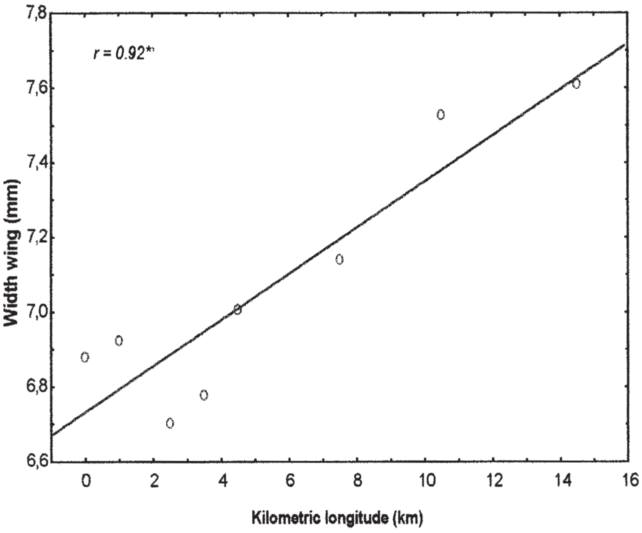

*Figure 3.* – The west-to-east variation of the wing width (stand means).

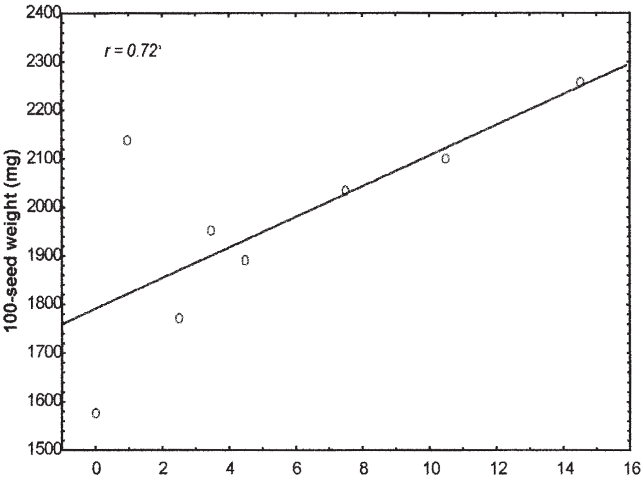

*Figure 4.* – The west-to-east variation of the 100-seed weight (stand means).

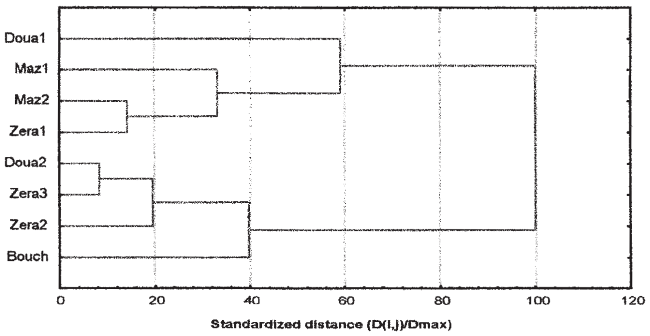

*Figure 5.* – WPGMA-tree clustering analysis (Euclidian distance).

Results from principal component analysis on 12 seed, cone and plantlet traits showed that more than 92% of the variance was explained by the four first components. Plans 1-2, 1-3 and 2-3 (Data non shown) permitted to detect a multitrait geographical pattern but Douaouda2 was atypical. This is a beach stand made up of a few dozens of trees.

Results from tree clustering analysis on the same traits are presented in *Fig. 5*. This analysis reinforce the pattern already detected by PCA. Two clusters were individualised comprising, for the first one, eastern stands (Bouchaoui, Zeralda2 and 3), except Douaouda2, and, for the second, western stands (Douaouda1, Mazafran1 and 2, Zeralda1). Thus, similarity between stands seems to be linked with spatial proximity, but random variation likely occurs in small stands.

## Discussion

Morphologic traits of seed and cone are informative even if they came from material collected in natural stands (DORMAN, 1976), because their heritability in place is high enough. Plantlet characters, since measured in a common environment, allowed us to acquire genetic information and to estimate genetic parameters.

Within the studied region, Aleppo pine stands were not greatly differentiated from each other. Relatively small distances between stands would allow substantial gene migration by pollen and/or seed, which should prevent any significant genetic differentiation to occur. Isolated trees here and there between stands could also be of importance for gene exchange. Yet, some traits, as cotyledon anisotropy or the number of cotyledons showed a more or less important spatial differentiation between stands. The hypothesis that some of the studied traits may be under strong maternal influence[4] can not be disregarded since gene flow for the female side (Seeds) will be shorter than from the male side (Pollen).

On the other hand, (family) variation within stands was notable for all seed, cone and plantlet characters. Family intraclass coefficient and/or heritability values were high, while coefficients of variation within families were low. This gives evidence for a heterogeneity among families and for a relative homogeneity within families. This between- and within-family variation pattern, may be due to the high genetic variance in the population and/or high levels of inbreeding in the seed collected (due to selfing as well as possible neighbourhood inbreeding). Both these effects are confounded and do not allow any firm conclusion about small scale genetic structure in these stands. However, this is speculative and requires further testing. High estimates of family variance may be obtained if

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[4] Such an influence needs to perform diallel matings to compare reciprocal effects.

mating mainly occur within small cohorts of trees in a population (BALIUCKAS *et al.,* 2000). This results in upward bias in estimating intra-class correlations and heritabilities (BOYLE and YEH, 1987), what could explain, in part, the high values obtained for these parameters.

At the region level, isolation by distance was not probably so complete, and common pools of genotypes would be shared among stands, hence the small stand differentiation. These stands are, likely to be parts of a same ancestor population, formerly more widespread, and, now essentially bordered on sites neglected by agriculture or maintained for protection purposes. However, parameters as size and location of stands may play a role in shaping genetic variation within stands. This variation was generally of lesser importance in small and/or coastal stands. Stands of small size may, eventually, differentiate by random drift (WRIGHT, 1969; WRIGHT, 1976); coastal stands, owing to their more or less linear configuration and the presence of the sea on one side, are less exposed to receive exogenous genes by migration (FALCONER, 1958).

Pot cultures of seedlings (underway) over a few years, should be informative upon the mode and the magnitude of genetic variation of juvenile characters presenting interest for early selection. Juvenile vigour, which is an important component of seedling aptitude to withstand competition following transplantation in the field, is generally intended. Whether the variation pattern observed for seed and plantlet traits was maintained at seedling stage, selection for juvenile vigour should concern the individual and family levels rather than stand level.

Given the size of the studied area (about 15 km long and 3 km large), it was not expected to find marked links between traits and geographic variables such as altitude or longitude; nevertheless, regression and correlation permitted to detect a west-east cline of variation for 100-seed weight and wing width, parallel with an increase in annual rainfall (628 mm/year, 694 mm/year and 717 mm/year at three localities from western to eastern parts of the zone). Yet, one can not be certain that there is a relation of cause and effect between these clines, especially as rainfall differences were not so large and as forest tree seeds from arid regions are generally heavier than those from rainy regions in forest trees (WRIGHT, 1976). This pattern of variation was not found in our case. In addition, weighted seeds were not sorted in any manner; so, we could not reject the idea that differences in 100-seed weight might also be due to more or less large quantities of empty seeds. And it just so happened, in forest trees, that proportions of empty seeds are always greater in inbred seed lots (ZOBEL and TALBERT, 1984). Inbreeding is more frequent in small stands (WRIGHT, 1976), and such stands (Douaouda1 and 2, Mazafran1) occurred in the western part of the region.

Contrary to univariate approach, multivariate methods allowed to detect structures of variation which suggest to separate eastern stands from western stands, but the separation was not perfect. The small sized sandy-soil coastal stand of Douaouda2 was, indeed, included within the group of eastern stands although it was thriving at the western side of the region. This asks the question of its wild or artificial nature. On the latter assumption, its close similarity with Zeralda2, an other sandy soil stand, may suggest that it could come from it.

Few papers deal with morphological variations in cone, seed and/or plantlet traits in Aleppo pine, especially at the within-region of provenance scale. An *in-situ* study of about 30 Algerian provenances, distributed from coast to saharian Atlas mountains, was carried out by KADIK (1986). It was shown that large phenotypic differences existed among provenances for cone traits and that seeds of provenances from arid regions were smaller and had widest wing than seeds of provenances from wet regions. These results at the whole species range level were similar to those found in our study. In Tunisia, a sample of 29 provenances allowed to highlight a strong geographic variability for 1000-seeds weight and cone weight; other traits such as cone dimensions or cotyledon numbers were less variable (KHOULA and SGHAÏER, 2000). In addition, it was found a variation of seed weight with altitude, provenances of lower altitudes having lighter seeds than their counterparts of higher altitudes. In regard to latitude and longitude, lighter seeds were found in northern and/or eastern populations. The (inverse) clines of seed weight variation with altitude and latitude can not be simply connected to a cline of pluviometry because rainfall is heavier in high altitudes and latitudes in North Africa. Other selective pressures ought to be involved in these variations. Let us remark that the altitudinal variation highlighted by KHOULA and SGHAÏER (2000) in Tunisia was in opposition to that found by KADIK (1986) in Algeria.

Elsewhere in the Mediterranean region, a study conducted in a clonal seed orchard of 76 *plus* trees from natural stands of Eubea Island (Greece) revealed a significant variation between clones in cone and seed traits. Notably, cone dimensions were found to be strongly heritable (MATZIRIS, 1998). Average 1000-seed weight recorded in this study was greater than those coming from provenance studies by authors as TOCCI (1979), PANETSOS (1981) or SCHILLER and WAISEL (1989); this was likely due to selection and/or to the break down of neighbourhood inbreeds which often occur in natural stands and cause inbreeding. But, site or management effects in the orchard could not be disregarded.

In experiments using populations from the whole range of the species, it was observed a significant geographic variability in seed, plantlet and seedling characters (PALMBERG, 1975, in south-eastern Australia; PELIZZO and TOCCI, 1978, in Italy). In Italy, there was no cline of variation depending on geographic variables such as latitude or longitude, while positive correlations with altitude for certain traits were detected in Australia.

Generally, wide tests of provenances all highlighted a large geographic variability for cone, seed and plantlet traits in Aleppo pine. In contrast, it seems that there was no substantial variability among stands within a same region of provenances in Aleppo pine when they were separated only by distances up to 15 km, as it was shown in this work. This finding in Aleppo pine for cone, seed and plantlet traits does not agree with those of WRIGHT *et al.* (1992) or SAENZ-ROMERO and GURIES (2002). In the former, it was observed significant genetic differentiation for seedling root size among stands separated by relatively short distances; in the latter, it was found that stands show significant differentiation for plantlet and seedling traits at geographic distances up to 25 km. So, such findings can not be generalized for all traits and all pine species.

As a direct implication for selection in Aleppo pine, it may not be of interest to retain stands or restricted areas when prospecting at a local scale; rather, priority should be given to selecting families and/or individuals to be tested on descendants, as long as good early criteria of selection are detected, which seems to be the case in this species (PELIZZO and TOCCI, 1978; KHOULA and SGHAÏER, 2000). The substantial family within-stand variability is a guarantee for buffering environmental changes. And, at the whole population level, because of the lack of differentiation among stands, it would be sufficient to preserve only a few of stands for ensuring efficient gene conservation.

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# Effect of Nitrogen, Phosphorous and Potassium Fertilizers on Growth of Stock Plants of *Tectona grandis* (Linn. f.) and Rooting Behaviour of Shoot Cuttings

By A. Husen and M. Pal

Plant Physiology, Botany Division, Forest Research Institute, P.O. New Forest, Dehra Dun-248006, India.
(Email: adroot92@yahoo.co.in)

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## Summary

The experiment was conducted to study the effect of nitrogen (N), phosphorus (P) and potassium (K) fertilizers on the growth of stock plants and rooting behaviour of coppice shoot cuttings of teak (*Tectona grandis* Linn. f.). Different combinations of N, P and K were applied to teak seedlings grown in earthenware pots. Growth observations on seedlings were recorded. Thereafter the seedlings were coppiced and data on coppice shoot growth were recorded in the month of June, 8-weeks after coppicing. Treatment of N, P and K fertilizers promoted the growth of stock plants. The highest concentration i.e., $N_{100ppm} + P_{100ppm} + K_{100ppm}$ caused maximum growth enhancement in terms of mean height, mean collar diameter and mean number of leaves produced on the stock plants. Fertilizers also increased the number of coppice shoots and their length. The coppice shoots were made into mono-nodal, leafy, softwood cut-