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# Electing superior trees: a study on the phenotypic traits of stone pine (*Pinus pinea* L.)

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*Abstract.* The purpose of this study was electing superior Stone Pine trees to get the seeds with high genetic traits. Methods. This study is carried out in four sites using four samples per site. Ten phenotypic traits of the trees in our sample are examined to determine the maximum value for each trait (measured in points out of ten). We then calculate the percentage value for each tree; weighted of each trait's value. Then, the total number of points for each tree is calculated in order to be able to choose trees that have achieved the highest points i.e. considered superior. As a result four superior trees are elected from each site. Next, a one-way analysis of variance (Anova) is conducted on the superior trees in each site. The results show no significant differences between them. However, when the analysis is applied to traits, statistically significant differences are found where some traits outweigh the others in terms of the role they play in the evaluation of superior trees, and thus we can adopt these traits as basic traits for evaluate the superior trees. Scientific novelty. According to this study, these superior trees can be selected as distinct mother seeds which can be used in propagation Pinus pinea.

Keywords: election, superior trees, stone pine, Anova, traits.

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### Introduction

Pinus pinea L. (Pinaceae), is one of the most characteristic tree species of the landscape because of its singular umbrella shape and the ancient use of its large, nutlike edible seeds for human consumption [1, p. 2]. The nut-bearing stone pine (Pinus pinea L.) is being a good source of unsaturated high quality fats, protein, vitamins, minerals, and bioactive compounds [2, p. 3]. Its pine nuts were already consumed by Neanderthals in the Middle Palaeolithic, before the last glacial maximum in [3, p. 359].

Stone pine is frequently preferred in afforestation practices due to its ecological, economic and aesthetical characteristics, and it is among the top species that provide important contribution to the national economy as a non-wood product [4, p. 416], [5, p. 161].

Turkey would have a potential to produce about 600,000 tons of cones and 15,600 tons of pine nut, which with current market prices would be worth 320 million to 550 million USD, respectively [6, p. 114]. In France the production of cones averages 3700 kg ha<sup>-1</sup> yr<sup>-1</sup>, or about 1200 kg ha<sup>-1</sup> yr<sup>-1</sup> of pine nuts. In Spain, trees with a large canopy are reported to have yielded from 1000 to 2000 cones tree<sup>-1</sup> [7, p.76].

The main importers of pine nut are Italy, Turkey, Qatar, Saudi Arabia, Brazil, and United States. In Lebanon, pine nut is recognized as the "white gold" among managers and tenants of stone pine forests [8, p. 122].

Cultivation of stone pine allows the forest owners getting revenues from annual cone yields even on lands not adequate for most agricultural crops [9, p. 669]. Stone pine is a tree that can be managed through thinning, pruning and grafting [10, p. 4].

Though current agronomic knowledge about stone pine as orchard nut crop is still limited, and most plantations continue to be managed as extensive forestry or agroforestry systems with trees grown from seeds without selected [11, p. 16].

No cultivars or cultivated varieties has been defined for stone pine yet, but recently, several elite clones and a clone mixture, selected for outstanding cone production, have been registered in Spain and Portugal for their grafted use in agroforestry systems or orchards [12, p. 79].

Selection is the first genetic improvement processes, this process means that a very small percentage of the population for one or more of the desired traits will be selected. The selection of an adapted population and seed trees inside them represents a chance to increase forest stability and productivity over the next generations of breeding [13, p. 2].

Phenotypic selection is a method of selection, which several trees in a group are selected according to their overall morphological characteristics or phenotypes. The external appearance of the individual is the first evidence of the selection process. The elector depends principally on the statistical probability which means that a good phenotype has a sufficient genetic background. The selection of superior trees is the first step to the success of improvement, and it has proven great effectiveness and unusual success in raising forest trees [14, p. 3].

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The superior tree is known as the recommended tree for production or breeding after classification, it has distinct properties such as the stem is vertical/straight/cylindrical in shape, without branching at the base (bottom), rapid growth and balanced crown, resistant to diseases and pests, resistant to drought and cold. So the goal of selecting the superior trees is to obtain large amounts of genetic profit quickly and cheaply. The similarity of the external conditions which surround the trees and the comparison of trees based on the selection criteria and the best of them are chosen as a superior tree. The advantage of this method is the selected tree showed the best characteristics under a similar environment to other neighbor trees and this increases the possibility that the differentiation of the desired traits is genetically related (according to the genetic nature of the individual) and the influence of the environment on this differentiation is canceled, this increases the chances of transferring the desired traits to the offspring [15, p. 283].

The superior trees of stone pines had been selected as plus trees by phenotypic traits, namely their outstanding cone yield and good environmental adaptation [16, p. 73].

There is a study conducted in China on Larix olgensis trees, where superior trees chosen based on number of criteria: the diameter at breast height, Tree height, branch angle, crown width, and Stem straightness degree [17, p. 1].

There is a study conducted on Ailanthus excelsa Roxb trees the selection was made on phenotypic assessment of desirable characters of economic interest such as stem straightness, selfpruning ability; clear bole height, low branching habit, disease resistance [18, p. 543].

While in other study growth rate and morphology were chosen as selection criteria for early generations in Pinus wallichiana improvement programs in order to aim of improving growth of this specie [15, p. 279].

Family selection is an effective means of tree genetic improvement, which is crucial for improving the yield, wood characteristics, and stress resistance of forest trees. Family selection is based on the phenotypic value of a family [19, p. 7].

In this light, purpose of the research is election of superior Pinus pine trees by studying the phenotypic characteristics, and comparison of the studied phenotypes of the selected superior trees.

### Methods

There are 4 different sites were selected in forest Pinus pine (AL, TA, RA, BR), four samples were selected within each site, the area of one or repeated sample is 400 m<sup>2</sup>. These selected samples are representative of the whole site as shown in table 1.

Table 1The symbol of the samples and the number of treesin the studied samples

Trees number	a	Ь	с	d
AL	40	36	40	38
TA	31	25	25	29
RA	31	33	30	30
BR	30	35	34	28

Within each sample, the following procedures and phenotypic characteristics were measured (for all studied trees):

\* Numbering (Marking) trees of samples: paints were used to mark each tree at all selected sites with a number, to recognize the trees, and to know the number of them within each sample.

\* Measuring the height of trees H: the height of all trees within the selected samples was measured by using an Altimeter for Measuring Height trees.

\* Measuring the diameters of trees DBH: the diameters of all trees within the selected samples were measured by using the cloth meter scale by measuring the circumference of the tree at breast height 1.3m and calculating the diameter value by dividing the circumference by  $\pi$ .

The relationship used:  $U = 2 \pi r$ ,

where U is the circumference of the tree, r is the radius of the tree.

\* Trunk Height Measurement Hs: the height of the trunk is the distance between the ground and the first major branch of the trunk, it was measured by using a height measuring device.

\* Stump straightness St: the straightness of the trunk was determined by giving the straightness values for each tree, it was compared the tree trunks with the index of straightening the trunk.

\* Weight of the fruiting cone CLw: after collecting the mature cones from the studied trees, the cones of each tree were weighted, to compute the average weight of the cone for each tree.

\* The average angle of the suspension of the first three lateral branches An: the angle which formed between the first three lateral branches and the main stem was measured manually by using a protractor, then it was calculated the average angle of the first three branches.

\* Average diameter of the first three lateral branches Db: firstly it was measured the circumference of the first three lateral branches by using a cloth meter scale, then it was calculated the diameter by dividing the circumference by  $\pi$ , finally the average of the measured branches diameters were computed.

\* Diminishing stem thickness DS: it was measured by using the following equation: DS = (DS1 - DS2)/L where:

DS: Diminishing stem thickness.

DS1: Diameter of minimum height (at breast height, 1.3 m). DS2: Diameter of maximum height.

L: the distance between the two measurement levels.

\* Crown width Cw: it was measured in two perpendicular directions by meter scale, then calculated the average.

\* Crown height Ch: Crown height is the distance between the first main branch of the stem and the top of the tree, it was measured by using an Altimeter for Measuring Height trees.

Comparing the values of the studied tree traits with the maximum value of the traits and selecting the superior trees.

The maximum value for each of the studied traits was initially determined at the level of each sample, the maximum phenotypic traits would be given 100 points (10 characteristics), so that each characteristic would take 10 points, then the percentage of the value characteristic of each tree was calculated from the maximum value of this characteristic (weighting), so the percentage of the value of the characteristic at the

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tree = (the value of the measured characteristic / the maximum value of the characteristic)  $\times$  100, according to this percentage this characteristic is given points out of ten-point, after calculating the points that each characteristic of the tree will obtain, we calculate the total number of points which achieved the described tree (the number of points out of 100 points), then it is possible to select the trees that achieved the highest points and consider them as the superior trees [20, p. 17]. It should be pointed out that the characteristic of the straightness of the trunk were calculated by using the guide of the straightness of the trunk as shown in table 2.

As for the straightness of the trunk was determined by giving the straightness values and comparing the tree trunks with the index of the straightness of the trunk as shown in table 3.

### Comparison of the studied phenotypes of superior trees

After we knew the superior trees (one tree for each sample), the data of the studied characteristics of the superior trees (ten traits) were entered during SSPS, we aim to compare the superior trees within one site and compare the studied traits to discover the degree of their influence and their weight in evaluating the superior trees.

#### Results

### 1. Selected Superior Trees

1.1. The superior trees on the site TA: the results for each tree in site TAa showed that the TAa30th tree was the better and had score 90 points. Fig. 1 shows the superiority of the TAa30th tree over the rest of the sample.

The superior trees on the site TA: the results for each tree in site TAb showed that the TAb18th tree was the better and had score 86 points. Fig. 2 shows the superiority of the TAb18th tree over the rest of the sample.

The superior trees on the site TA: the results for each tree in site TAc showed that the TAc1st tree was the better and had score 96 points. Figure (3) shows the superiority of the TAc1st tree over the rest of the sample.

The superior trees on the site TA: the results for each tree in site TAd showed that the TAd23th tree was the better and had score 94 points. Fig. 4 shows the superiority of the TAd23th tree over the rest of the sample.

1.2. The superior trees on the site AL:

\* The results for each tree in site ALa showed that the ALa40th tree was the better and had score 88 points.

\* The results for each tree in site ALb showed that the ALb36th tree was the better and had score 86 points.

\* The results for each tree in site ALc showed that the ALc40th tree was the better and had score 86 points.

\* The results for each tree in site ALd showed that the ALb37th tree was the better and had score 86 points.

1.3. The superior trees on the site RA:

\* The results for each tree in site RAa showed that the RAa18th tree was the better and had score 88 points.

\* The results for each tree in site RAb showed that the RAb9th tree was the better and had score 86 points.

\* The results for each tree in site RAc showed that the RAc3rd tree was the better and had score 88 points.

\* The results for each tree in site RAd showed that the RAb4th tree was the better and had score 88 points.

1.4. The superior trees on the site BR:

\* The results for each tree in site BRa showed that the BRa16th tree was the better and had score 94 points.

\* The results for each tree in site BRb showed that the BRb25th tree was the better and had score 90 points.

\* The results for each tree in site BRc showed that the BRc-1st tree was the better and had score 82 points.

\* The results for each tree in site BRd showed that the BRb28th tree was the better and had score 84 points.

2. Results of comparing studied phenotypic of superior trees:

2.1. Comparing the phenotypes of superior trees at the site AL: When we carried out analysis of variance ANOVA by using the statistical SPSS program between the superior trees at the site AL as shown in table 4, it found that there were no significant differences between the superior trees. Therefore, superior trees can be considered equal in their superiority at the site AL.

When carrying out analysis of variance ANOVA for the studied traits, it found that there were significant statistical differences  $P \le 0.05$ , and the LSD test results showed the following:

# Table 2 Scoring according to the studied characteristics

		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
Character	0-30 % of MAX	< 30–50 % of MAX	< 50-70 % of MAX	< 70–90 % of MAX	< 90 % of MAX
Height (H)	2	4	6	8	10
Diameters (DBH)	2	4	6	8	10
Trunk Height (Hs)	2	4	6	8	10
Weight of the fruiting cone (CLw)	2	4	6	8	10
Average angle (An)	2	4	6	8	10
Diameter of the first three lateral branches (Db)	2	4	6	8	10
Diminishing stem thickness (DS)	2	4	6	8	10
Crown width (Cw)	2	4	6	8	10
Crown height (Ch)	2	4	6	8	10

### Table 3 Scoring according to the trait St

Stump straightness (St)	5	4	3	2	1
Score	2	4	6	8	10



### Number of tree











### Fig. 3. The scores for each tree in TAc sample

\* There are no significant differences between the traits (H, DBH, An, CLw, St, Cw, Ch) among them, therefore they can be considered as similar traits in degree of their impact on the evaluation and classification of superior trees.

\* It was noted that there were significant differences between the traits (H, DBH, An, CLw, St, Cw, Ch) and (Hs, Ds, Db), where (H, DBH, An, CLw, St, Cw, Ch) were superior to (Hs, Ds, Db).

\* It was noted that there were significant differences between the traits (Hs, Ds, Db), where trait (Hs) was superior to (Ds, DB), and trait (Ds) was superior to (Db).

\* In this light, it can be said that traits (H, DBH, An, CLw, St, Cw, Ch) have the most influential and weight on the evaluation of superior trees then the trait (Hs), (Ds) respectively, and the trait (Db) has the least weight and the effect on the evaluation of superior trees. Biology and biotechnologies





Table 4

The po	ints o	f ch	aracteri	istics	superio	r tree	at the	? site	Al	-
										_

	Score studied traits											
Superior trees	Height (H)	Trunk Height (Hs)	Diameters (DBH)	Diminishing stem thickness (DS)	Average angle (An)	Diameter of the first three lateral branches (Db)	Weight of the fruiting cone (CLw)	Stump straight- ness (St)	Crown width (Cw)	Crown height (Ch)		
Ala40	10	8	10	8	10	2	10	10	10	10		
ALb36	10	8	10	6	10	2	10	10	10	10		
ALc40	10	8	8	6	10	4	8	10	10	10		
ALd37	10	8	10	6	10	2	10	10	10	10		

## Table 5

	Score studied traits												
Superior trees	Height (H)	Trunk Height (Hs)	Diameters (DBH)	Diminishing stem thickness (DS)	Average angle (An)	Diameter of the first three lat- eral branches (Db)	Weight of the fruiting cone (CLw)	Stump straight- ness (St)	Crown width (Cw)	Crown height (Ch)			
TAa30	10	8	8	10	8	8	8	10	10	10			
Tab18	10	8	8	6	10	8	8	10	8	10			
TAcl	10	10	10	10	10	6	10	10	10	10			
Tad23	10	10	10	8	10	6	10	10	10	10			

Table 6

The points of characteristics superior tree at the site RA

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		Score studied traits											
Superior trees	Height (H)	Trunk Height (Hs)	Diameters (DBH)	Diminishing stem thickness (DS)	Average angle (An)	Diameter of the first three lateral branches (Db)	Weight of the fruiting cone (CLw)	Stump straightness (St)	Crown width (Cw)	Crown height (Ch)			
RAa18	10	6	10	10	10	6	10	10	10	10			
RAb9	10	8	10	6	8	4	10	10	10	10			
RAc3	10	8	8	10	8	8	8	10	8	10			
RAd4	10	10	8	8	10	6	8	10	8	10			

	Score studied traits											
Superior trees	Height (H)	Trunk Height (Hs)	Diameters (DBH)	Diminishing stem thickness (DS)	Average angle (An)	Diameter of the first three lateral branches (Db)	Weight of the fruiting cone (CLw)	Stump straightness (St)	Crown width (Cw)	Crown height (Ch)		
BRa16	10	10	10	10	8	6	10	10	10	10		
BRb25	10	8	10	8	10	4	10	10	10	10		
BRcl	10	10	10	4	6	2	10	10	10	10		
BRd28	10	10	8	8	8	4	8	10	8	10		

Table 7 The points of characteristics superior tree at the site BR

2.2. Comparing the phenotypes of superior trees at the site TA: When we carried out analysis of variance ANOVA by using the statistical SPSS program between the superior trees at the site TA as shown in table 5, it found that there were no significant differences between the superior trees. Therefore, superior trees can be considered equal in their superiority at the site TA.

When carrying out analysis of variance ANOVA for the studied traits, it found that there were significant statistical differences  $P \le 0.05$ , and the LSD test results showed the following:

\* There are no significant differences between the traits (H, Hs, DBH, Ds, An, CLw, St, Cw, Ch) among them, therefore they can be considered as similar traits in degree of their impact on the evaluation and classification of superior trees.

\* It was noted that there were significant differences between the traits (H, Hs, DBH, An, CLw, St, Cw, Ch) and (Db), where (H, Hs, DBH, An, CLw, St.Cw, Ch) were superior to (Db).

\* While there were no significant differences between (Ds) and (Db). Therefore, it can be said that the traits (H, Hs, DBH, Ds, An, CLw, St, Cw, Ch) have the most influential and weight on the evaluation of superior trees and it found that trait (Db) has the least weight and the effect on the evaluation of superior trees.

2.3. Comparing the phenotypes of superior trees at the site RA: When we carried out analysis of variance ANOVA by using the statistical SPSS program between the superior trees at the site RA as shown in table 6, it found that there were no significant differences between the superior trees. Therefore, superior trees can be considered equal in their superiority at the site RA.

When carrying out analysis of variance ANOVA for the studied traits, it found that there were significant statistical differences  $P \le 0.05$ , and the LSD test results showed the following:

\* There are no significant differences between the traits (H, DBH, Ds, An, CLw, St, Cw, Ch) among them, therefore they can be considered as similar traits in degree of their impact on the evaluation and classification of superior trees.

\* It was noted that there were significant differences between the traits (H, DBH, Ds, An, CLw, St, Cw, Ch) and (Hs, DB), where (H, DBH, Ds, An, CLw, St, Cw, Ch) were superior to (Hs, DB).

\* It was noted that there were significant differences between the traits (Hs, Db), where trait (Hs) was superior to (Db).

\* In this light, it can be said that traits (H, DBH, Ds, An, CLw, St, Cw, Ch) have the most influential and weight on the evaluation of superior trees then the trait (Hs), and the trait (Db) has the least weight and the effect on the evaluation of superior trees.

2.4. Comparing the phenotypes of superior trees at the site BR: When we carried out analysis of variance ANOVA by using the statistical SPSS program between the superior trees at the site BR as shown in table 7, it found that there were no significant differences between the superior trees. Therefore, superior trees can be considered equal in their superiority at the site BR.

When carrying out analysis of variance ANOVA for the studied traits, it found that there were significant statistical differences  $P \le 0.05$ , and the LSD test results showed the following:

\* There are no significant differences between the traits (H, Hs, DBH, CLw, St, Cw, Ch) among them, therefore they can be considered as similar traits in degree of their impact on the evaluation and classification of superior trees.

\* It was noted that there were significant differences between the traits (H, Hs, DBH, CLw, St, Cw, Ch) and (Ds, Db), where (H, Hs, DBH, CLw, St, Cw, Ch) were superior to (Ds, Db).

\* It was noted that there were significant differences between the traits (H, St, Ch) and trait (An), where the traits (H, St, Ch) were superior to (An).

\* It was noted that there were significant differences between the traits (An) and trait (Db), where the trait (An) was superior to (Db).

\* It was noted that there were significant differences between the traits (Ds) and trait (Db), where the trait (Ds) was superior to (Db).

\* In this light, it can be said that the traits (H, Hs, DBH, CLw, St, Cw, Ch) have the most influential and weight on the evaluation of superior trees then the trait (An), and the trait (Db) has the least weight and the effect on the evaluation of superior trees.

### **Discussion and Conclusion**

1. After studying the phenotypes (H, Hs, DBH, Ds, An, Db, CLw, St, Cw, Ch), It was selected four superior trees at the site AL (Ala40, ALb36, ALc40, ALd37), (TAa30, TAb18, TAc1, Tad23) at the site TA, (RAa18, RAb9, RAc3, RAd4) at the site RA, and (BRa16, BRb25, BRc1, BRd28) at the site BR. According to this study, these superior trees can be selected as distinct mother seeds which can be used in propagation Pinus pinea. 77

2. After comparing the superior trees within each site, it was found that there is no significant differences between them, while the comparing the studied traits showed that the traits (H, DBH, An, CLw, St, Cw, Ch) have the most influential and weight on the superior trees which were evaluated, then

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trait (Hs) and (Ds) respectively, therefore the less influential traits can be excluded for the evaluation of the superior trees and give more focus on the most influential traits, thus we can adopt these traits as basic traits for evaluate the superior trees.

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