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הופק בתאריך: 11.01.00

דו"ח דיווח מדעי

קוד זיהוי : 274-0030-99

נושא המחקר: ברירה וטיפוח מחטניים

סוג דו"ח : מדעי שנתי

חוקר ראשי : שילר גבריאל

חוקרים משניים:

מקורות מימון עבורם מיועד הדו"ח:

קרן קיימת לישראל

תקציר הדו"ח:

במשך שנת 1999 התמקד המחקר בחלקות הנסיון בהם נטועים אקוטיפים (גנוטיפים) שונים של אורן ירושלים אשר נטעו בשנת 1989 ביתיר ובבית-דגן.
נבדקה השונות הגנטית באוכלוסיות של אורן ירושלים הגדלות באזורים שונים סביב אגן הים התיכון: ביוון, איטליה, ספרד ומדינות צפון אפריקה וגם ישראל. בישראל נדגמו היערות הטבעיים והם הכרמל, ירקא ובית-ג'אן אשר על הר מירון. כמו כן נדגמו האקוטיפים אשר גדלים בחלקת הנסיון ביער יתיר.
נבדקה ההתנהגות הפסיולוגית של אקוטיפים שונים של אורן ירושלים אשר נטועים ביתיר ובבית-דגן. מוצא האקוטיפים מיוון, הכרמל, אלג'יריה, בית-ג'אן וירקא.
התוצאה המרשימה ביותר שנמצאה בבדיקות של השונות הגנטית היא, שכנראה כתוצאה מברירה שנוצרה במשתלה חמה (גילת), נטיעה בשטח שעל גבול המדבר כלומר, עקת מים רבה, וברירה על ידי היערנים (דילול), חל שינוי משמעותי בשונות הגנטית של האוכלוסיה. העצים שנשארו בחיים ביתיר הם הטרוזיגוטיים במידה רבה לעומת זו הקיימת באוכלוסיות המוצא השונות. תוצאה זו יש לה השלכות על אסטרטגית איסוף הזרעים במדינה למטרות יעור. רצוי לשקול את האפשרות שעצי עלית ביער יתיר יהוו את מקור הזרעים עבור נטיעות אורן ירושלים במדינה.
תוצאה שניה חשובה בתחום הפיסיולוגי היא שהאקוטיפ היווני בהשואה לאקוטיפים אחרים שנטועים ביתיר, מצליח לקיים פוטנציאל מים פחות שלילי וממשיך לקיים חילופי גזים, טרנספירציה ופוטוסינטזה, אמנם ברמה מאוד נמוכה אך זאת לעומת חוסר כל פעילות באקוטיפים האחרים.

חתימות ואישורים:

חוקר ראשי	מנהל המחלקה	מנהל המכון	אמרכלות	תאריך
שילר	[signature]	[signature]	אמה רזין [signature]	17.1.2000

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FORDAPT

12 months report

September 1998 - September 1999

Second Progress Report

for the period September 1998- September 1999

Submitted by:

PARTICIPANT: IL 5 - A.R.O., ISRAEL

Gabriel Schiller, Leonid Korol and Nir Atzmon.

During the last 12 months, the activities can be described as follows:

Task 1.	**Characterization of genetic diversity.**
Subtask 1.3	**Molecular characterization of genetic diversity.**
1.3.1	**Geographic genetic diversity**
	Isozymes:

Materials and Methods:

Genetic diversity analysis using isoenzyme starch gel electrophoresis started February 1998. Analysis were performed using 8 megagametophytes per single tree of the trees selected in the provenance trial at the Yatir forest and in the other natural Aleppo pine forests in Israel, using the procedures according to Conkle *et al*,. (1982), with several adjustments according to the expertise in our laboratory. Genetic diversity analysis of over-see populations were done using 100 megagametophytes per popularion. Populations analyzedare shown in Table 1.

Results:

The Italian and the Jordanian populations were not yet analyzed. The Telagh population although analyzed was not included in the statistical analysis because only 8 trees of this origin are growing at Yatir; such a low number of trees can not be analyzed statistically.

Allele frequencies, scored from the stained gels, were subjected to statistical analysis implementing the Biosys-1, a computer program for the analysis of allelic variation in genetic (Swofford and Selander, 1981).

Using Nei's (1972) analysis of genetic identity, Figure 1 shows the similarity between the populations analyzed. The similarity among all the twenty populations analyzed is very high. Still, the twenty populations are divided into three main groups, i.e. French, Spanish, Moroccan and two of the four Tunisian populations are connected on one brunch. The Greece populations whether natural or planted at Yatir on a separated brunch. All the Israeli natural and planted populations with the Italian population of Otricoli planted at Yatir, and except of the Mt. Carmel population planted at Yatir, are grouped on the third brunch.

Using Nei's (1972) analysis of genetic distance, Figure 2 shows the very small distance between all the twenty populations analyzed. The grouping of the populations is identical to the grouping shown in Figure 1.

Using Distance Wagner procedure, Figure 3 shows a different grouping of the populations analyzed. Although the general distance from root is not large, still four groups of populations are being formed very close to the root of the diagram. All the North African populations, except two from Tunisia, and including Spanish and French populations and including one natural Israeli population are on the one brunch. All the Israeli populations natural and planted including the Italian population at Yatir, with the exception of the Mt. Carmel population at Yatir are on the second brunch. All the Greek population are on a separated brunch; and two Tunisian and one Israeli population are also on a separated brunch.

Using Cavalli-Sforza and Edwards (1967) chord distance, which is a better measure for the phylogeny, Figure 4 shows again the division of the populations analyzed into three brunches which differ in some aspects from the former figures. One brunch include the European and Moroccan populations, the second brunch include all the Israeli native and planted populations together with the Tunisian populations. The third brunch is composed of all Greek populations.

The amount of within populations excess or deficiency of heterozygotes (Hs) is shown in Figure 5. The figure shows a most interesting phenomenon, the populations planted at Yatir forest have excess in heterozygots whereas, the origin populations show deficit in heterozygots.

Discussion:

The results gained in the present analysis of diversity, strengthen the conclusions presented in a former study (Schiller *et al*, 1986) on the genetic relations and phylogeny of Aleppo pine range-wide populations around the Mediterranean. In our analysis too, it is evident that Israeli natural populations are related to North African, i.e., Tunisian populations. The existence of a strong relations between the Moroccan, Algerian and West European populations; and the separation of the Greek populations is evident also in the present analysis.

The second important result, shown in Figure 5, is the drastic change or the turn over of the heterozygosity within population. It seems as if the forestry practice and the very harsh ecological conditions at the Yatir forest had facilitated the rise in heterozygosity.

Task 2 Mechanisms of drought response

2.1. Ecophysiological mechanisms of drought response

(Physiological differences between the ecotypes in relation to drought resistance).

1. **Controlled experiment** - The main task was to produce seedlings (by vegetative propagation) from the different ecotypes as shown in Table 2 of the first report, and to test their physiological response during withholding irrigation.

Results: The growth rate of the shoots during the year 1998 was very low due to the low rainfall in winter 1997/98, and the unusual hot summer of 1998. Because of the climate in this winter (1998/99) which was very mild and very dry winter (the most driest winter in the last 30 years), the budsd did not stop their scars growth; there was not enough units of cold temperature, needed to facilitate rooting. This has inflicted on the rooting capacity, and in spit all our efforts to vegetatively propagate trees of several provenances, we failed again.

2. **Field experiment** - The main task is to study the physiological activity of the trees in the provenance trail at Yatir forest during the different seasons with special regard to their activity during the dry season.
Transpiration and photosynthesis are measured by ADC, LCA3 infra-red gas analyzer. The ADC was carefully studied and measurements started during August. These measurements are followed by xylem (= needle) water potential measurements using the pressure chamber according to scholander *et al.* (1965).

Results: During winter of 1998/99 rainfall was measured at Yatir, at the Northern boarders of the Negev dessert, and at Bet Dagan, in the central coastal plain of Israel,. Figure 6 show the rainfall events distribution and amounts at Yatir and Bet Dagan. The winter of 1998/99 was the most driest one since more than thirty years. At Yatir, only 140.5 mm and at Bet Dagan only 244.1 mm rainfall were measured.; whereas the mean annual rainfall at Yatir is 299 mm, and at Bet Dagan about 600 mm. At Yatir there is also loess soil which prevent water penetration, which further reduced the water availability to the trees.

Therefore, as shown in Figure 7 and 8, the xylem water potential of Aleppo pine trees growing in the provenance trials at Yatir and Bet Dagan are very low in comparison with the potentials achieved in regular years. At no time of measurements, Xylem water potential of trees in Yatir was always higher than -2.32 MPa, which has been established by Melzack *et al.* (1985) as the threshold potential for withholding transpiration and photosynthesis. At Bet Dagan, due to the higher rain amounts and the sandy soils more water was available to the trees, therefore xylem water potentials were higher most of the time which enabled higher physiological activity.

Photosynthesis and transpiration were measured at Yatir and Bet Dagan on several trees of each of the four different provenances at several dates. Average photosynthesis, transpiration and water use efficiency per provenance were calculated, Analysis of variance was conducted with site (Bet Dagan or Yatir) and Provenance (Three provenances common to both sites) in the model as categorical variables, and varius continuous covariates that were found to be statistically significant. For transpiration, site was highly significant, whereas provenance was not significant. The results are presented in Table 2 show that there are differences between provenances in transpiration rate, net photosynthesis and therefore water use efficiency.

Disscusion:

Differences among provenance planted at Yatir in height and diameter growth were presented in the first annual report. In a earlier study, significant differences between species and within species among provenances growing at the same site, were found in xylem water potentials and water saturation deficit of needles (Grunwald and Schiller, 1988). Also in the present study, there are differences in xylem water potentials between provenances growing at Yatir and at Bet Dagan; there are significant differences between provenances in water use efficiency. This differences are the probably the cause for the differences in the growth rate between the provenances analyzed. Interestingly, the pattern of differences between provenances found in Yatir differ from the one found in Bet Dagan. For instance, Telagh (Algeria) planted in Bet Dagan showed highest values in all parameters tested (transpiration, photosynthesis and water use efficiency) and lowest Yatir. On the other hand Ellea (Greece) showed the opposite pattern. This provenance showed the lowest values when planted in Bet Dagan and highest in Yatir. Those differences might be indicators for provenances adaptation to different sits. More study is needed to define the role of these differences.

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Table 1: The populations analyzed

-Caireval, France.	-Istiaia (A_3), Greece.
-Kassandra (A_5), Greece.	-Velez Blanco (A_{18}), Spain.
-jaraful (A_{20}) Spain	-Selloum (A_{10}), Tunis
-Birino, Tunis.	-Oum Djeddour (A_9), Tunis.
-Takrouna, Tunis.	-Tamaga, Morocco.
-Ikherifene, Morocco.	-Lalla Mimouna, Morocco.
-Vico del gargano (A_{27}), Italy.	-Larauto, Italy.
-Jerash/Ajlun, Jordan.	

-Mt. Carmel, Israel, (natural, earlier seed collections, after fire regeneration).

-Mt. Carmel, Israel (natural).	-Mt. Carmel, Israel, (planted at Yatir).
-Bet Jann, Israel (natural)	-Bet Jann, Israel, (planted at Yatir). Yirka,
-Israel (natural)	-
	-Ellea (A_2), Greece, (planted at Yatir).
	-Otricoli (A_{26}), Italy, (planted at Yatir).
	-Telagh (A_{31}), Algeria, (planted at Yatir).

*Letters and numbers in parenthesis refer to the FAO-IUFRO-seed collection, 1976.

Genetic variability at 25 loci in all populations
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(standard errors in parentheses)

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity Direct-count	Mean heterozygosity HdyWbg expected**
1. Caireval(FR)	43.0	1.4	20.0	.042	.054
	(.0)	(.1)		(.020)	(.022)
2. N.Euboia (GR)	76.0	2.0	52.0	.138	.188
	(.0)	(.2)		(.036)	(.041)
3. Kassandra (GR)	45.0	1.9	48.0	.126	.177
	(.0)	(.2)		(.037)	(.038)
4. Velez Blanco(SP)	36.0	1.3	20.0	.051	.055
	(.0)	(.1)		(.028)	(.024)
5. Jarafuel (SP)	72.0	1.4	16.0	.045	.059
	(.0)	(.1)		(.021)	(.024)
6. Selloum (TUN)	43.0	1.5	32.0	.085	.111
	(.0)	(.1)		(.033)	(.034)
7. Birino (TUN)	72.0	1.5	32.0	.059	.076
	(.0)	(.1)		(.025)	(.025)
8. Oum Jedour (TUN)	65.0	1.5	36.0	.059	.097
	(.0)	(.1)		(.021)	(.027)
9. Takrouna (TUN)	57.0	1.6	36.0	.088	.129
	(.0)	(.1)		(.030)	(.036)
10. Tamga (MOR)	17.0	1.3	20.0	.049	.067
	(.0)	(.1)		(.028)	(.027)
11. Ikherifene (MOR)	34.0	1.4	20.0	.036	.068
	(.0)	(.1)		(.017)	(.024)
12. Lalla Mimouna(MO	16.0	1.2	20.0	.025	.052
	(.0)	(.1)		(.013)	(.024)
13. Charauto (IT)	108.0	1.8	44.0	.137	.186
	(.0)	(.1)		(.039)	(.046)
14. Vico del Car.(IT	108.0	1.8	44.0	.144	.180
	(.0)	(.2)		(.037)	(.043)
15. Jerash (JO)	167.5	1.6	44.0	.110	.156
	(.3)	(.1)		(.039)	(.039)
16. Ajlum (JO)	54.3	1.4	32.0	.121	.123
	(1.0)	(.1)		(.045)	(.037)
17. Yirka/Israel	37.0	1.5	36.0	.124	.118
	(.0)	(.1)		(.042)	(.035)
18. Bet Jan/Israel	27.0	1.3	12.0	.049	.054
	(.0)	(.1)		(.024)	(.027)
19. Turky (TUR)	30.0	1.4	44.0	.103	.151
	(.0)	(.1)		(.033)	(.038)
20. Carmel Nat. (IS)	238.9	1.8	44.0	.116	.142
	(.4)	(.1)		(.029)	(.033)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

** Unbiased estimate (see Nei, 1978)

Figur 1: Similarity among the populations analyzed

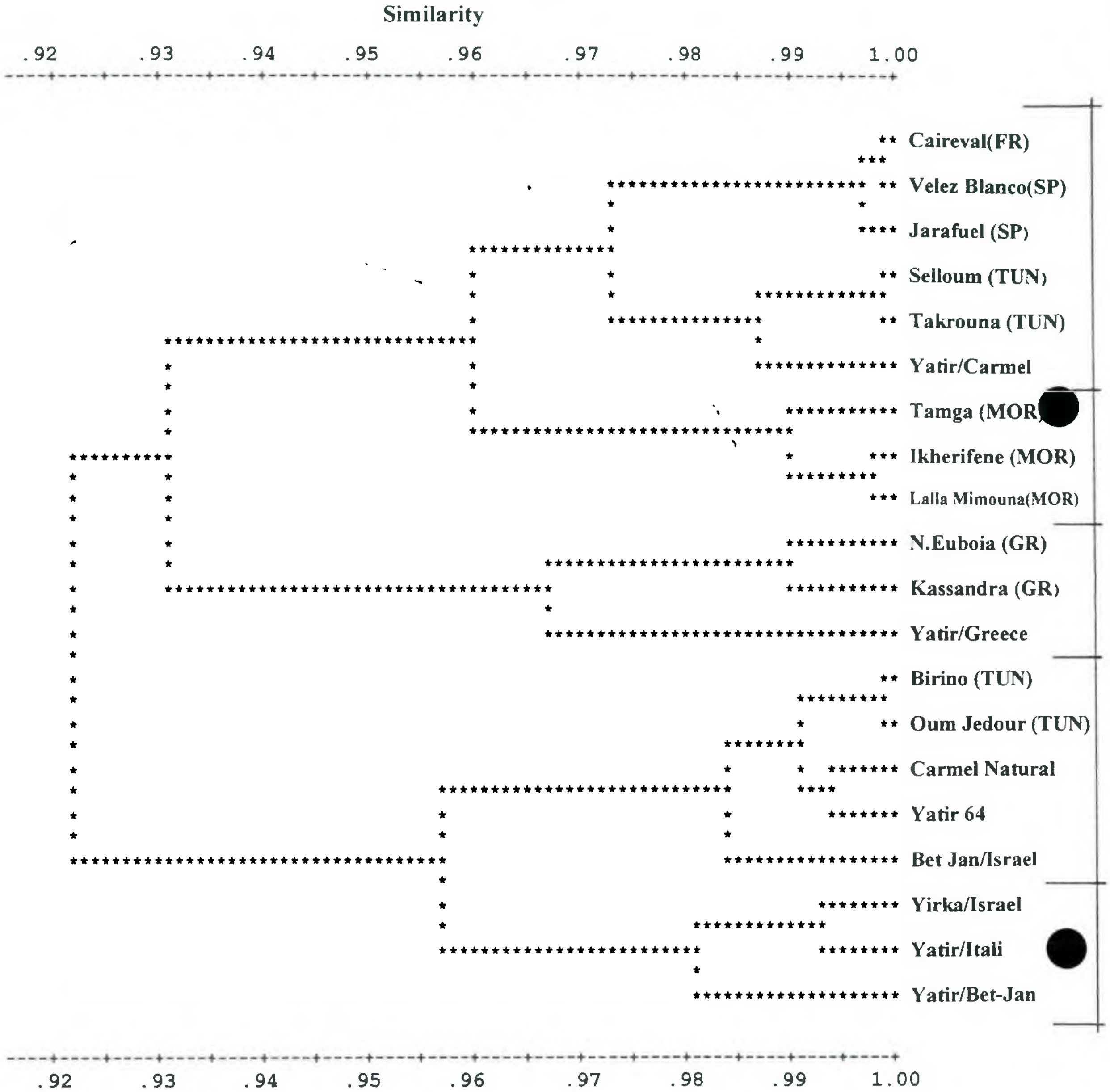


Figure 2: The Distance among the populations analyzed.

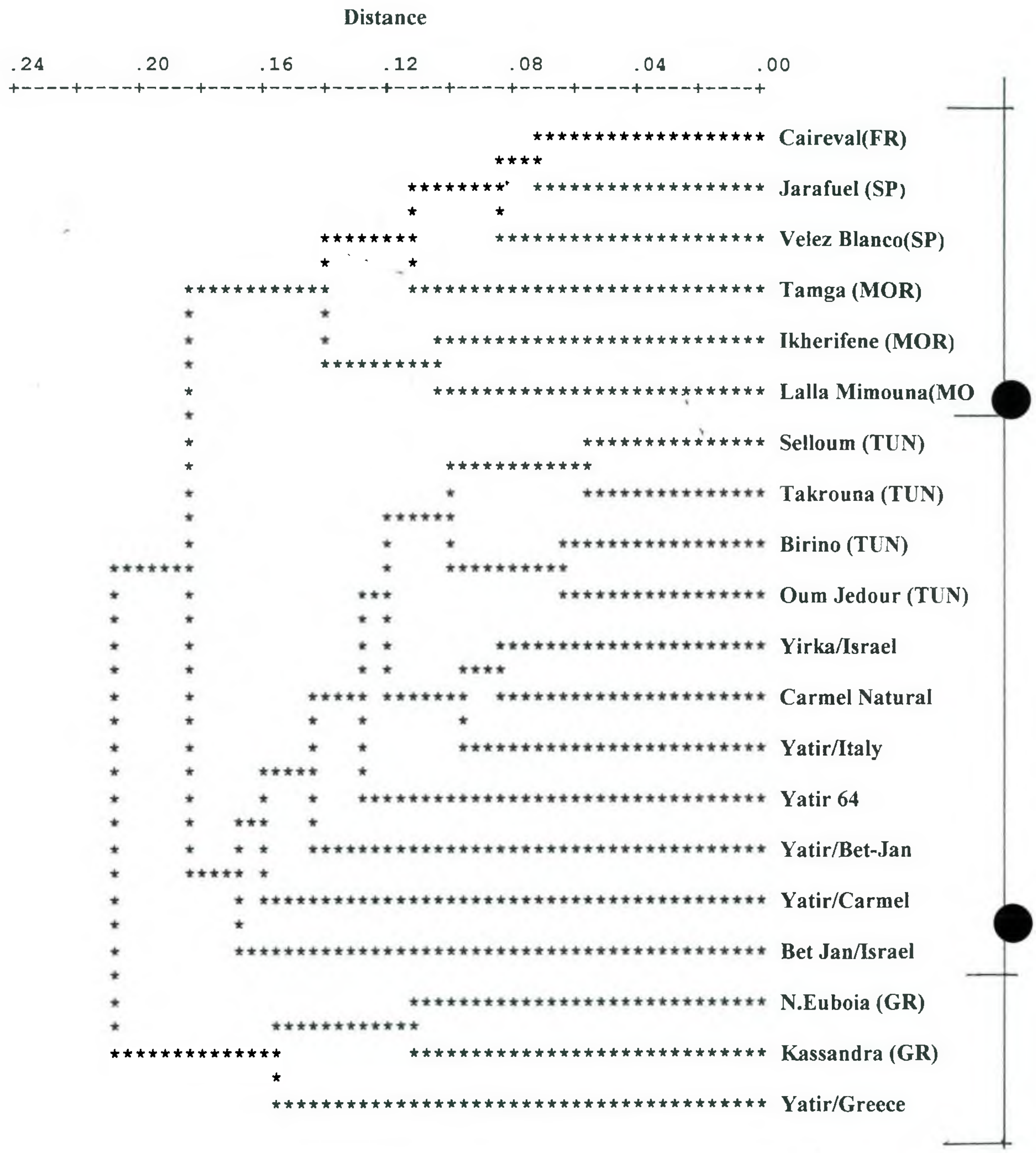
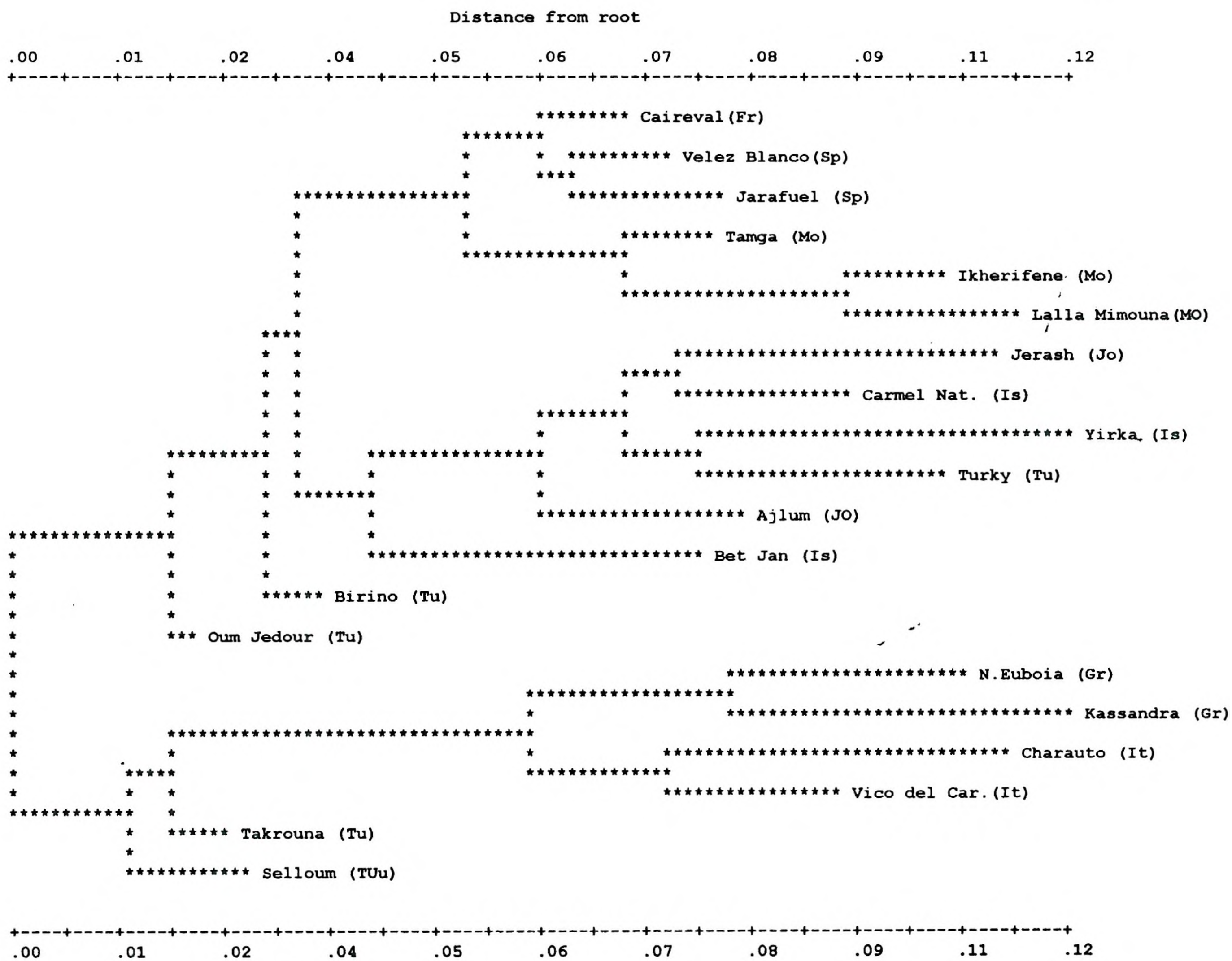


Figure 3:

Wagner tree produced by rooting at midpoint of longest path



Total length of tree = .650

Figure 4:

Coefficient used: Cavalli-Sforza & Edwards (1967) chord distance

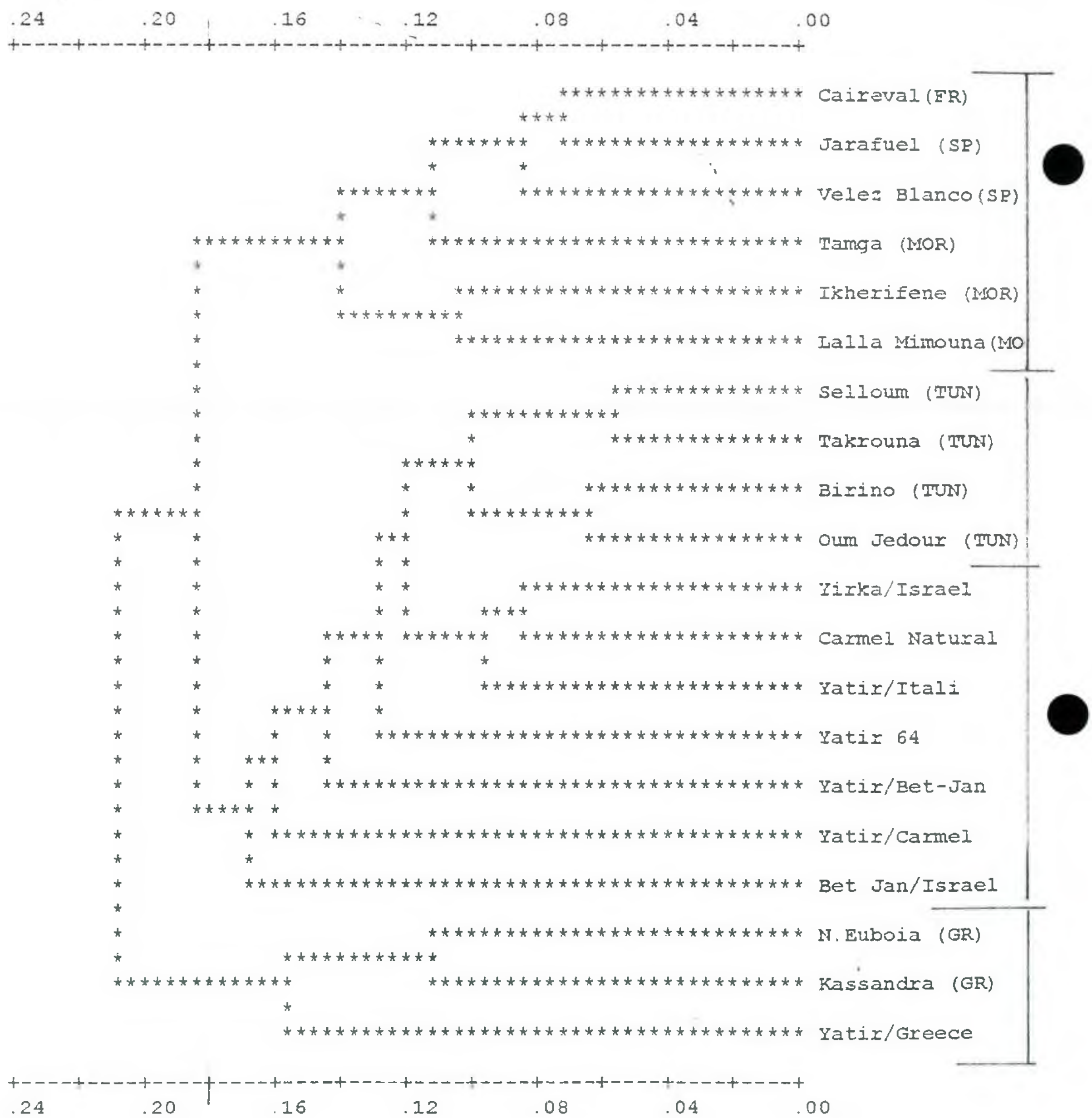


Figure 5: **Index Heterozygote Defficiency or Excess**

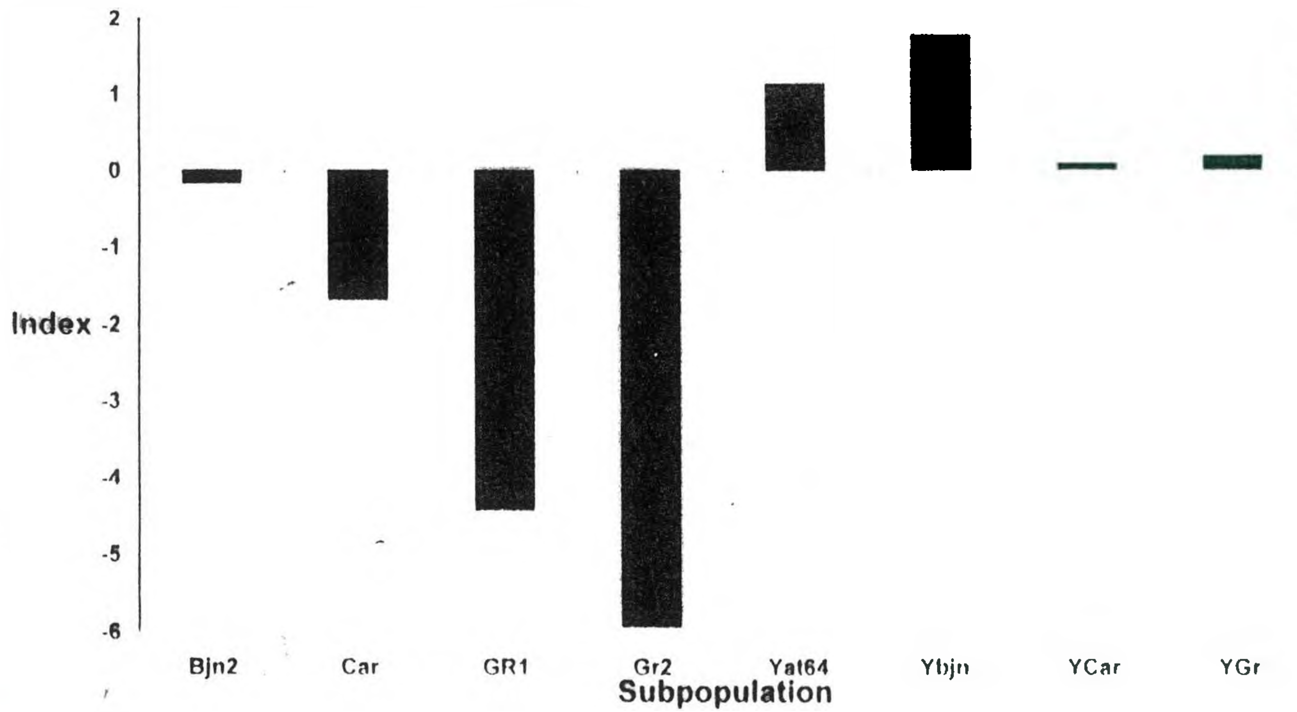


Table 2: Between and within sites (Bet Dagan and Yatir) analysis of variance of transpiration, photosynthesis and water use efficiency

Transpiration (millimol/m^2/s)

Source	Least Sq Mean	Prob. >F
Provenances: at Bet Dagan		0.3483
Otricoli, Italy	0.6105	
Telagh, Algeria	0.6192	
Ellea, Greece	0.5971	
Mt. Carmel (Elkosh), Israel	0.6163	
Provenances: at Yatir		0.6578
Telagh, Algeria	0.3888	
Mt. Carmel	0.4084	
Ellea, Greece	0.4134	
Bet Jann, Israel	0.4012	
Sites (Bet Dagan or Yatir		0.0001
Provenances at both sites		0.8045
Bet Dagan	0.5285	
Yatir	0.4607	

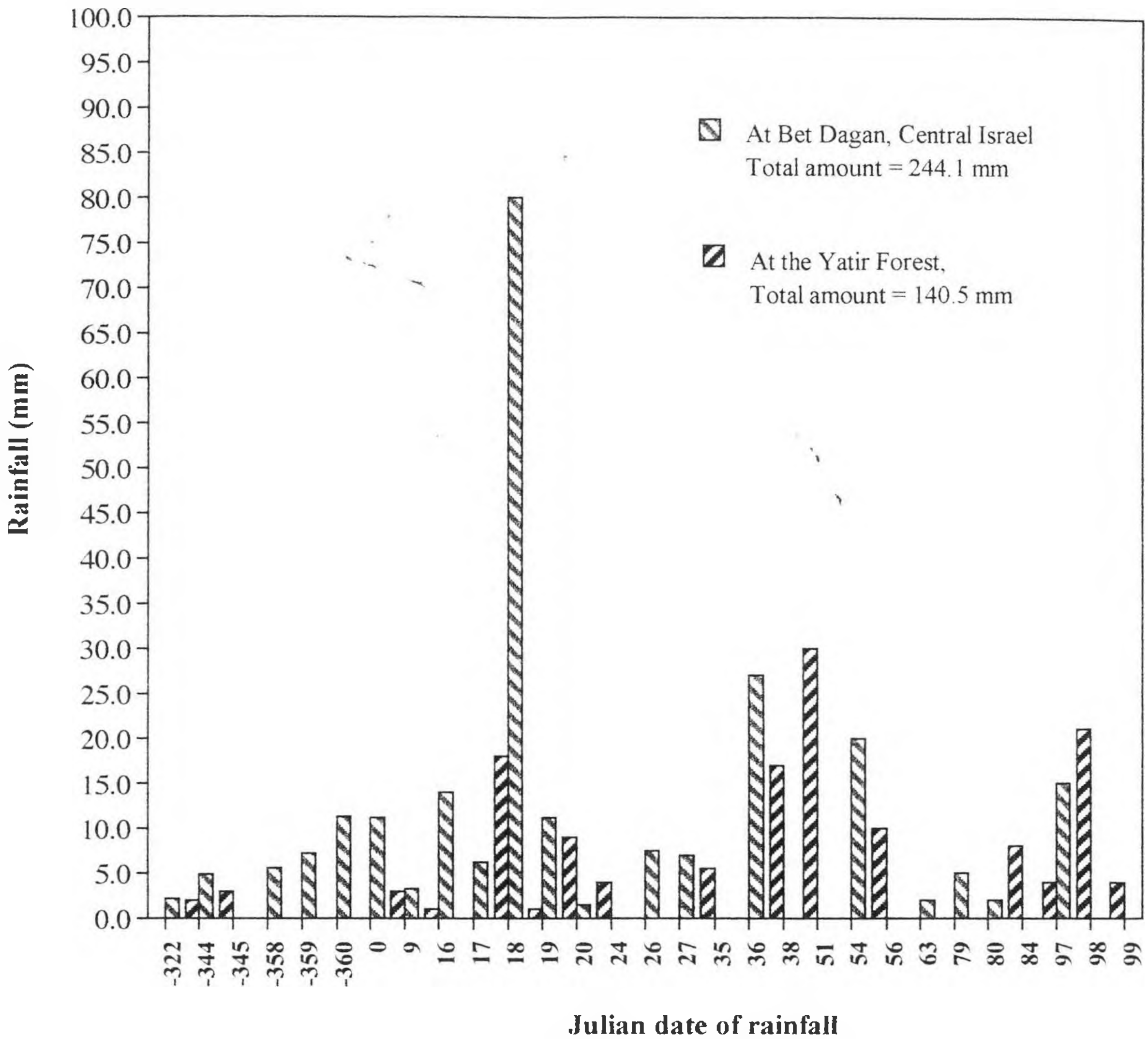
Photosynthesis (micromol/m^2/s)

Source	Least Sq Mean	Prob. >F
Provenances: at Bet Dagan		0.0711
Otricoli, Italy	3.0544	
Telagh, Algeria	3.2149	
Ellea, Greece	2.9307	
Mt. Carmel (Elkosh), Israel	2.8075	
Provenances: at Yatir		0.0001
Telagh, Algeria	1.2204	
Mt. Carmel	1.2174	
Ellea, Greece	1.6680	
Bet Jann, Israel	1.4011	
Sites (Bet Dagan or Yatir		0.0001
Provenances at both sites		0.0078
Bet Dagan	2.9161	
Yatir	1.3600	

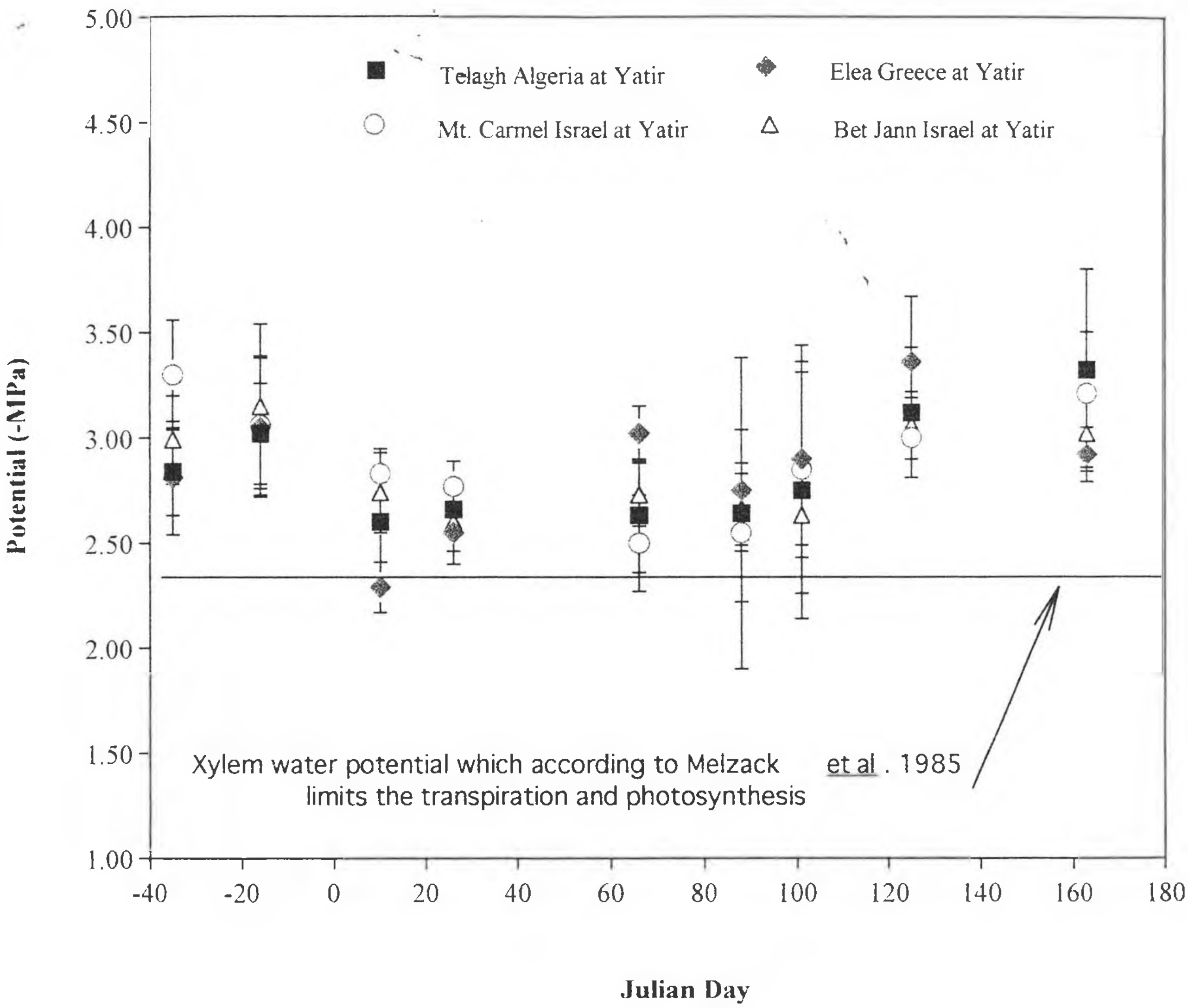
Water use efficiency

Source	Least Sq Mean	Prob. >F
Provenances: at Bet Dagan		0.0374
Otricoli, Italy	4.4514	
Telagh, Algeria	4.7820	
Ellea, Greece	4.7190	
Mt. Carmel (Elkosh), Israel	4.0152	
Provenances: at Yatir		0.0008
Telagh, Algeria	2.1064	
Mt. Carmel	2.2394	
Ellea, Greece	3.0765	
Bet Jann, Israel	2.7704	
Sites (Bet Dagan or Yatir		0.0001
Provenances at both sites		0.0088
Bet Dagan	5.4375	
Yatir	2.7850	

Rainfall at Bet Dagan and Yatir during the winter of 1998-99.



Xylem Water potentials at the end of 1998 and during 1999 of four different provenances planted at Yatir



Xylem water potentials at the end of 1998 and during 1999 of four different provenances planted at Bet Dagan