GENETIC IMPROVEMENT OF KANINJOT VARIETY FROM CLONAL SELECTION

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Abstract:

The "Kaninjot" cultivar, autochthonous and widespread is remarkable for high percentage and good quality of the oil. It has dual use. It has periodical production, but the variability that this genotype possesses has been exploited through clonal selection to find individuals with high production constant, oil percentage and bigger average fruit weight. The selection underwent two phases: In the first phase: over a population of 2583 trees, in a five year period, where each year the trees that were qualified had inflorescence and fructified, while the others were considered out of use. In the second phase for 17 clone candidates derived from selection of the first phase production evaluation was done for kg/trees/ year, (i) oil percentage % fresh matter done through Soxhlet, (iii) average fruit weight (g), (iv) production periodicity estimated through Pearce and Dobersek-Urbank. Each clone displayed differently its genetic potentials, with differences for production constant. Constant production had 6 individuals (0.00 – 0.30). The variability for fruit weight was from 0.26 g up to 1.24 g more than the standard (12 individual). For the oil percentage 6 individuals with 0.1 up to 2.1% more than the average of the population were selected. In the final evaluation there resulted to be: (i) 12 clones with superior PC. (ii) 6 clones possess superior for constant production and oil percentage (PC+OP). (iii) **6** clones possess the three selection features; constant production, oil percentage and fruit weight *(PC+Fw+Op*). The selected individuals have differences as far as the pomological and technological characteristics are concerned.

Key words: Clone; Olive oil; Cultivar; rooting capacity; Selection; Olea europaea.

Introduction

The study focused on *Kaninjot cv*, which occupies 50% of the oliviculture surface, cultivated all over the country. It has high percentage of oil (27%) and average fruit weight of (3.6 g). On the other hand it is remarkable for its periodic production, up to the degree that it makes its cultivation inefficient.

Within the population of this variety there are individuals which have a variety of productions, with uniformity and higher percentage of oil. This fact has enabled its selection within its population, by always evaluating the profitable genotype to make them favour the new oliviculture and to improve the efficiency in a farm.

The study of the pomological criteria makes up the basis of classification and the characterization of the individuals within the Kaninjot cv. in three different areas. Clonal selection went through a 12-year-period 1995-2007, in three fields of the population Kaninjot cv, (Panaja, Oshtime, Jonufer) with 2583 trees (Figure-1).

Material and Methods

SELECTION OBJECTIVE: The variability of trees has been exploited to select: (i) *individuals with a high production constant (PC=0.0-0,20). (ii)* oil percentage 1,5-2% higher than the average of the population. (iii) Fruit weight over (0,2 g).

The selection "in bulk" in accordance with the phenotype was based on the outer appearance for the qualitative features (fruit form, symmetry, resistance to *Cycloconium oleaginum*, etc) whereas for the quantitative features (fruit weight, production etc). The selection underwent three phases (2+5+5 years): (Figure-2).

2.1. **SELECTION BASE***:* first massive selection: (two years) *in three fields of the population Kaninjot cv, (Panaja, Oshtime, Jonufer) with 2583 trees.* Only the trees with inflorescence and that fructified.

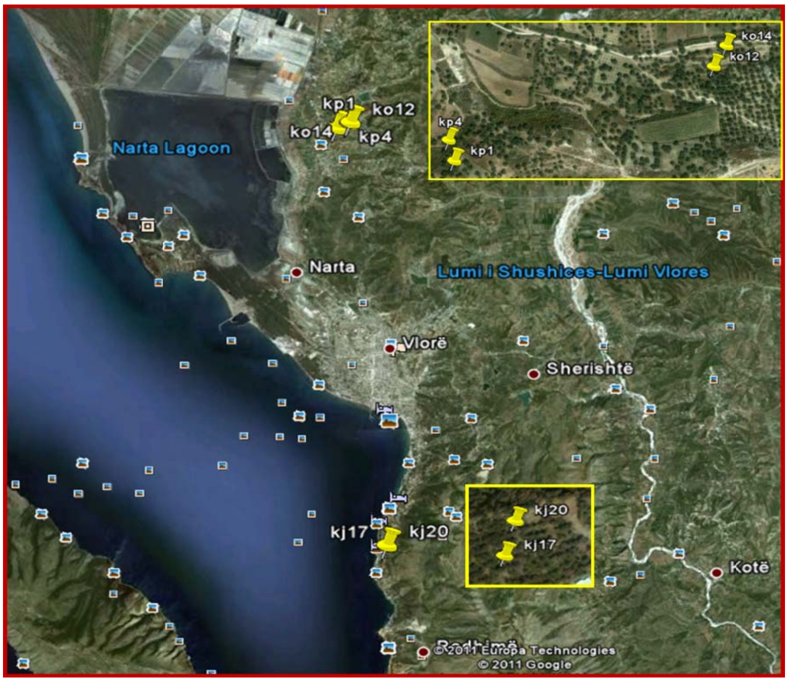

Figure 1- The population and clones of Kaninjot cultivar in Jonufra, in Panaja and Oshtima

Figure 2-The Selection scheme.

BASE SELECTION (2 years)	**AGRONOMIC SELECTION (5 years)**	**CERTIFICATION (5 years)**
Selection of population	*Pomologic and Technologic valuation*	*Genetic and Sanitary*
Selection of trees	*Selection of candidate*	

2.2. **AGRONOMIC SELECTION**: (five years): in each year, which classified the trees that had inflorescence and fructified [1, 15].

2.3. **CERTIFICATION (genetic and sanitary):** (five years) Clone candidates were analyzed each year for the quantitative and qualitative characters which prove qualitative changes. (i) tree production kg/tree/year, (ii) oil percentage, analyzed over a quantity of paste 5 kg/trees/year, estimated in % of fresh matter, (Soxhlet, extracted with ether petrol). (iii) average fruit weight calculated over 100 fruit of each tree/year and **(iv)** proportion between pulp and endocarp (P/E) [3, 15].

(iv) Periodicity Coefficient (**PC**), calculated by *Pearce and Dobersek-Urbank* over: production/tree/year, according to the formula**;**

$$I = \sqrt{\frac{1}{n-1}\sum_{i-2}^{n}\left(\frac{P(i)-P(i-1)}{P(i)+P(i-1)}\right)}$$

where n- represents the number of samplings or the weighing and P(i) year production (i) [11, 13].

- ***A hundred fruit***, fully ripen, were taken during the pickings period in each area. They were weighed, their dimensions were calculated (L) and (l) and the ratio (L/l) flowering; length, the number of the flowers, fertile flowers, fruit set during picking. The tree, its power form and volume were evaluated. Production for each tree was weighed in kg.

- **Percentage of oil in fruit**. Oil extraction was carried out through the soxhlet method in high temperature through ether petroleum as

an organic solvent (according to the chemical laboratory Olive Research Institute (ORI), oil percentage compared to fresh matter and in (%) of dried matter, peroxide analysis, acidity [2, 6].

- ***Definition of acidity (%):*** 5-10 ml oil is dissolved in 50-150 ml (ether petroleum + ethyl alcohol), some drops of phenolphthalein with alcoholic dip of KOH 0,1N until the pink colour remains for 30 sec. The percentage of acidity was calculated through the formula:

$$X = \frac{a \times 0.0282 \times 100}{p}$$

where: (a)- the quality of the alcoholic dip of KOH 0,1 N (ml), (0.0282)- the quantity in grams of the oleic acid, which corresponds to 1ml dip KOH 0,1N, (p)- weight of the sample in grams.

-***Selection of the individuals***. Clones with differences from the average of the population were evaluated and tested, primarily for constant production in the values 0.0-0.2 of the periodicity coefficient (PC), as well as oil percentage and average fruit weight [2, 13].

The results were analysed each year and for each tree in order to prove the variability, closeness and distance from the varietal standard.

- ***Certification sanitary***: the unknown sanitary status of clones in University and IAM-Bari, Italy

2.4. Statistical analysis: Clones with differences from the average of the population were evaluated, tested and analyzed in JMP version 2010. (*Statistical Analysis Software 2008),* primarily for constant production in the values 0.0-0.2 of the Periodicity Coefficient (PC), as well as oil percentage and average fruit weight **[7, 9]**.

3. Results and Discussion

The 12 year results proved a great variability in the population of the Kaninjot cv. The best trees were selected systematically (each year), changed, done primarily by massive selection and later by individual evaluation.

3.1. MASSIVE SELECTION: There has been a great variability among the 870 trees analyzed during the first year of the massive selection, derived from a great population of 2583 olive trees (Table- 1).

Table 1- Basic population and massive selection of Kaninjot cv.

Nr	Representative Population	Nr. Trees Selection base I+II year	Nº qualified trees (*Agronomic selection*)				
			I year	**II year**	**III year**	**IV year**	**V.th year**
1.	**Panaja**	335	190	39	18	8	6
2.	**Oshtime**	1513	410	213	111	19	5
3.	**Jonufer**	735	270	90	37	13	6
	Selection dynamics	2583	870	342	266	40	17

At the end of the agronomic selection the best trees of each plot comprise only 1.9% of the base trees. By dynamically applying the same “selection” criterion, *(39%) were selected* during the second year*, (30.5%) during the third year, (4.5%) during the fourth year. At the end of the first phase definitely, the candidate clone trees were 17, which comprise 1.9% of the initial population. Only these trees had inflorescence and fructified each year and are considered to be of low periodicity level.*

Only (1.9%) of the trees had variability from the average of the population, because they have inflorescence and fructified homogenously every year. These trees were studied individually to guarantee safety for variability [11].

3.2 THE EVALUATION OF CANDIDATES CLONES. The results of **candidates** clones analyzed for five years for qualitative and quantitative characteristics have proved qualitative differences, not only in comparison with the varietal population, but also among each other. [13].

THE CALCULATED TOTAL PRODUCTION. (TP) the individual efficiency of each tree in each year has presented great variance. The total production of kg/tree realized in five years was different, (from 150 kg up to 360 kg). The production volume has been a function of the fructifying surface of the tree and the level of its regularity (Table-2). Because production is the outcome of the metabolic activity as a biological unity of the tree the individuals; KJ17, KO13, KO14, KP5 had bigger global production. In the cv KO13 big production has mainly been a function of the crown’s volume and not the cause of its regularity [6, 12].

PRODUCTION CONSTANT (PC)*:* The regularity of production has undergone changes even according to (P.C) “***marker***” has shown the level of the ability of the trees to yield at constant rates. The trees have had constant production where the value of the periodicity coefficient (PC) has been within the values (0.0-0.2). Following this logic only 6 individuals, (KP1, KP2, KP3, KP4, KO12, KJ20) have had constant level of production because they had values of their PC: (0.10, 0.17, 0.18, 0.20, 0.13, 0.27) [10, 14] (Table 2).

The PC value of the clones results to have considerable changes compared to the average of the standard population. (0.60), A lot of c. clones had a higher PC value than (**0.2**). From this point of view 3 candidates clones had PC values of (0.3-0.45), and 8 candidates clones between more than 0.45. The trees with a PC value of (0.0-0.2) have a really constant production. They present obvious changes over the other candidate clones that have a value of more than (0.2) of the constant production (PC) but, with the value of the standard population, despite the fact that they derive from the same population and are cultivated within the same environment [2, 8].

Table 2- Five-year-data : the population of C clones, for Production, Fruit, and fresh Oil %.

Plot C. Clone	Kg yield per Individual/year					Pr. Glob	P.C	Weight, Gram		Oil % l.fr.	(+)more than stand		
	I	II	III	IV	V	(Kg)		Fr	End		Fruit (g)	Oil (%)	(P.C)
KP1	40	40	35	35	40	190	0.10311	4.09	0.43	29.0	0.49	2.0	0.50
KP2	50	45	50	50	45	240	0.17921	3.88	0.5	27.3	0.28	0.3	0.43
KP3	50	50	60	65	80	275	0.18371	3.97	0.44	29.1	0.37	2.1	0.42
KP4	30	15	25	40	80	190	0.27494	4.84	0.56	28.2	1.24	1.2	0.33
KP5	60	40	150	5	35	290	0.63947	3.16	0.48	26.0	-	-	-
KP6	70	15	80	30	70	265	0.50186	3.67	0.52	26.9	0.07	-	0.10
KO12	50	30	45	50	80	255	0.20746	4.18	0.56	28.7	0.58	1.7	0.40
KO13	150	30	110	0	80	360	0.65903	3.68	0.58	23.9	-	-	-
KO14	80	90	70	25	10	305	0.32809	4.04	0.52	25.0	0.44	-	0.28
KO15	70	70	80	15	50	285	0.43175	3.86	0.56	29.2	0.26	2.2	0.17
KO16	60	70	78	12	30	250	0.68528	4.1	0.59	24.5	0.41	-	-
KJ17	120	80	100	35	90	425	0.32871	4.11	0.56	24.3	0.51	-	0.28
KJ18	60	20	110	10	40	240	0.60960	3.16	0.58	24.9	-	-	-
KJ19	60	30	50	70	20	230	0.46314	3.98	0.57	24.0	0.38	-	0.14
KJ20	50	60	40	35	50	235	0.13573	4.2	0.54	25.0	0.42	-	0.47
KJ21	70	20	35	15	80	220	0.48884	3.34	0.47	25.5	-	-	0.12
KJ22	50	40	50	0	10	150	0.66458	3.2	0.49	25.7	-	-	-

Individual choice has enabled the separation of a group of superior trees for *Periodicity coefficient* (PC), and finally the separation from this group of the most profitable trees. At this moment, after the 10th year, the possibility of varietal improvement according to this method has come to an end, because of lack of further change. In the trees selected as "**clones"** we were able to prove that productivity level was constant throughout all the years and we distinguished no statistical changes. In this case the phenomenon of their production makes up a genetic character, and the perennial choice based on a big population has completely eliminated those trees with harmful genes, as far as periodicity coefficient is concerne [11, 13, 14].

The value of the periodicity coefficient, analyzed through hierarchical clustering average (fig), for the level of distance; had variability which fluctuated from 0.10 (KP1), to 0.27 (KJ20), and orientated candidate clones in 4 homogenous groupings: (FIGURE-4)

- Accessions with Periodicity Coefficient (PC) 0.10 up to 0.30. Which are characterized of a lot of constant productions. (Annual production).
- Accessions with Periodicity Coefficient (PC) 0.30 up to 0.45 which are characterized of a constant average productivity.
- Group with Periodicity Coefficient (PC) more than 0.45 (PC) characterized for no constant productivity.

The variability of the trees constitutes not only a shift of the genetic equilibrium, but also its evolution within the population of the Kaninjot cultivar. By means of analytical evidence of the change of the "clone" individuals, genetic composition has been different from the trees of the parent population. Through clonally selection the quantitative features represented by the production expressed through periodicity coefficient (PC) ensure us of the inheritance of their features [3, 4]. On the other hand, the number of the accessions that differ from the varietal type has ensured us that we have great avoidance from the real values of the population. The reasons of avoidance are related to inner genetic causes and to the level of their relationship to the environment. Avoidance from the varietal standard for periodicity coefficient (PC) expressed through the letter sigma (σ), and analysed with the formula: $\sigma = \sqrt{\frac{\sum\left(x - \bar{x}\right)^2}{n-1}}$ where: (x)-any value; (X)-average value, (n)- nr of variables; and (n-1)- number of variables minus one.

It has shown the dispersion level in the population of Kaninjot cv where the quantitative features of the majority of the individuals stand nearby the average value (μ) of the population. By interpreting this dispersion, it can be seen that more than 76.2 % of the values can be found in the limit of μ±1σ of the average: in the limits of μ±2σ there are

95.1% of the values and in the limits of ±3σ, there are 99% of the values (Figure 6).

The variance analysis estimated over the features of; periodicity coefficient (PC), Oil %, Fruit weight, has characterized an interacting variance, which has been expressed in the phenotype changes as an effect of the interaction of the genotype to the environment. The PC change from one candidate clone to another, found each year comprises the phenotypic value of the production constant and through analysis, for the level of distances they were classified into three homogenous groupings [5, 8] (Table-2).

In a final analysis of the main index of research (PC), it was proved that only a small part of the population was selected (1.9%), which expressed in percentage gives the selection coefficient. The perennial results of the average value of the PC of the c clones and their change from the arithmetic average of the population confirmed the selection difference [4, 7].

FRUIT CALIBRE; The efficiency of the clone trees was related to the fruit weight as well as the biological variability of the tree in environmental conditions. [Figura-**4**]. Indices of the efficiency are fruit weight, the proportion of pulp/endocarp, (P/E) and the number of fruits in the inflorescence. We have considered average weight as a secondary requirement, and the level of variability has fluctuated from 3.12 gr to 4.84 g. There were several individuals with high average weight which have a dominant status in proportion with the others (*Tukey-cramer, lsd 2.12, q=0.05*).(Figure-3, 4). Trees with the nr. KP1, KP4, KO12, KO14, KJ17, KJ20, have changes with arithmetical average (3.6g). but although there were 13 c. clones dominant over the average value, only one had variable weight (bigger), with a value of 4.84 gram; which means 1.24 gram more than the varietal average. Candidate Clone (KP4) with average fruit weight 4.84 gr, had changes and great distance compared to the varietal standard (Table- 2).

OIL PERCENTAGE. Analysis to find individuals that possessed stabilized features of high oil percentage positioned them differently compared to Periodicity Coefficient (PC). The oil percentage proved analytically each year has had and still has a stable character [15].

The close points between oil percentage and fruit weight in each identity, analyzed bivariate density ellipsis P=99., evidenced constant individuals with oil percentage and high fruit weight. In *Figure-6*, within the plot we distinguish the variable accessions which possess oil percentage and fruit weight above the average during the five research years. The individuals that have these two characters not accompanied in pairs were positioned out of the ellipsis [1].

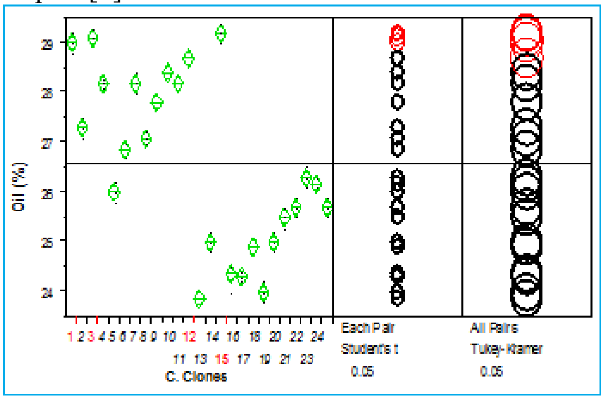

Figure 3- Anova testing for the Oil percentage

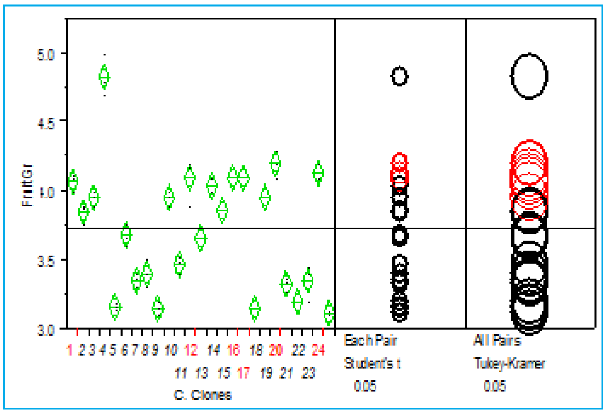

Figure 4- Anova testing for the Weight of fruits

THE PROPORTION PULPE/ENDOCARP, is a varietal characteristic, whereas in our case there were minor differences. Only the Clones KP4 and KJ17 had obvious differences compared to the others.

3.3 CERTIFICATION: The analysis to find dominance of the basic feature periodicity coefficient (PC) stabilized, as well as their positioning to secondary features: oil percentage (op) and average fruit weight (fw), has tied and positioned candidate clones differently. The correlative analysis distinguished the close points and the level of distance and selected the accessions with different PC, OP and FW to the varietal standard, which comprise a stable character [2, 4].

The Important role of native clones selection in Vlora, the unknown sanitary status of clones (IAM-Bari, Italy , with reference to virus and virus – like diseases and the identification of healthy clones to be used as immediate source for propagation, were the bases to undertake this work. Two out of 17 candidate clones olive samples tested by dsRNA analysis, resulted infected, whereas, 15 samples did not showed any presence of dsRNAs (replicative form virus

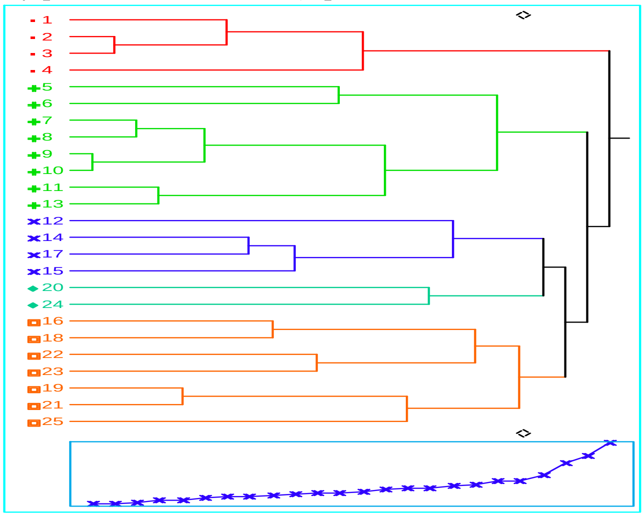

Figure 5- Dendogram hierarchical clustering for the similarity Of the C. clones for the ratio (PC).

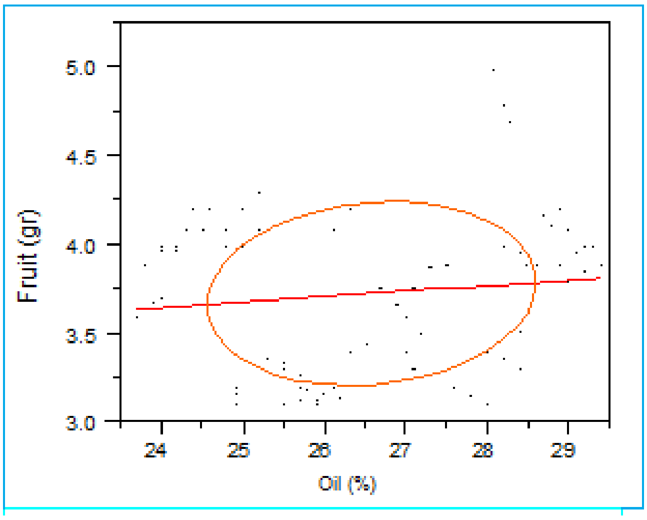

Figure 6- Positioning of c .clones for oil %, and average fruit weight.

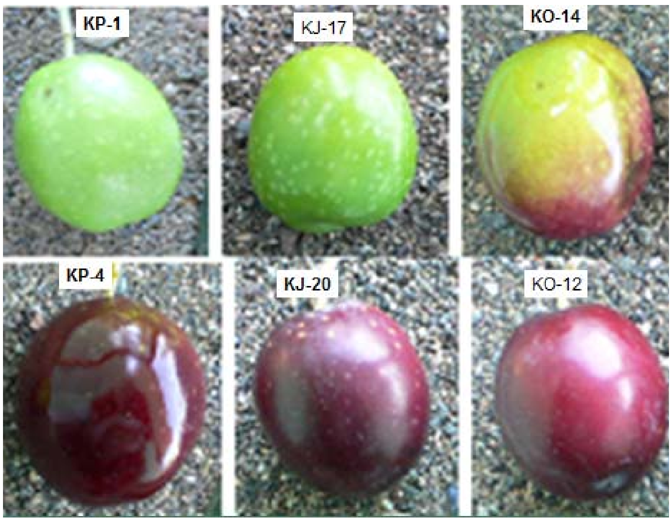

Figure 7- Six clones certified and selected

4. Conclusions

The 12 years selection of the Kaninjot cultivar, based on the results of the analysis certified the differences within the varietal population, the definition of similar features and the level of the distance between each other.

Within the Candidate Clones there were differences for the *Periodicity coefficient*, *oil percentage* and *average fruit weight*. There resulted to be (i) clones that possess *Periodicity coefficient* (***PC***) in superior levels: **12** (ii) clones that possess *Periodicity coefficient* and *oil percentage* (***PC+OP***): **6** (iii) clones that possess *Periodicity coefficient, oil percentage and fruit weight* ***(PC+Fw+Op):*** **6** clones (Table-2, Figure-7).

Within the population of candidate clone **(CC)** 6 individuals that possess a correlation of the three features: **KP1** ($40^0$32'32''N : $19^0$28'27''E); **KP4**($40^0$32'33''N : $19^0$28'27''E); **KO12** ($40^0$32'37''N : $19^0$28'45''E) **; KO14** ($40^0$32'38''N : $19^0$28'46''E); **KJ17** ($40^0$24'04''N : $19^0$28'52''E); **KJ20** ($40^0$24'05''N : $19^0$28'53''E); were certified and selected (Figure-1, 7).

The increase of efficiency and quality of new oliviculture must take into consideration the composition of the vegetative material of the olive and the inclusion in the structure of new olive-groves of the selected clones, which must represent the Kaninjot cultivar.

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