DESARROLLO DE METODOS PARA LA SELECCION DE PORTAINJERTOS DE AGUACATE EN ISRAEL (Desarrollo de métodos para estudios de investigación en campo a gran-escala en árboles frutales: La selección de portainjertos de aguacate en Israel como caso de estudio)[1]

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RESUMEN. Durante el presente siglo, el aguacate (*Persea americana* Mill.) se ha desarrollado como un cultivo frutal comercial en algunos países, incluyendo a Israel. La práctica es injertar cultivares en portainjertos. Se ha encontrado que los árboles son muy sensibles a algunos estrés del suelo, pero diferentes portainjertos varían en su sensibilidad. Muy poca información ha estado disponible concerniente al efecto del portainjerto en el desarrollo, resistencia y productividad del árbol injertado. Un estudio a gran-escala en campo pareció ser esencial para colectar la información requerida y para el posterior desarrollo exitoso del cultivo.

El presente articulo está dedicado a las técnicas desarrolladas en el trayecto del proyecto a gran-escala en el cual 700 experimentos de campo se han instalado desde 1968, en húertas comerciales. Portainjertos provenientes de semilla fueron utilizados en el primer juego 350 experimentos, que involucraron 120,000 árboles, y en el segundo juego de 350 experimentos se utilizaron portainjertos clonales, donde se usaron 60,000 árboles. Los experimentos se establecieron en cooperación con los productores y se utilizó un diseño simple. Se dio seguimiento a cada experimento cerca de 10 años: síntomas de estrés; tamaño del árbol, medido por fotografía aérea como proyección del área de la copa en el suelo; y rendimiento, como es cuantificada por los productores.

Los cálculos y análisis estadísticos fueron realizados para rendimiento acumulado, área ocupada por el árbol, "eficiencia del árbol" -en rendimiento por unidad de área, y alternancia en la producción. La uniformidad de cada grupo de árboles fue calculado el C.V. (coeficiente de variación). La selección negativa de portainjertos y fuentes del injerto, basadas en los resultados experimentales, condujo al mejoramiento del material de propagación utilizado, desde el comienzo de la investigación. Al final del primer juego de experimentos, cerca de 40 portainjertos superiores fueron recomendados para uso comercial, y cerca de 100 nuevos portainjertos clonales fueron desarrollados de árboles sobresalientes.

Estos últimos fueron evaluados en el segundo juego de experimentos.

PALABRAS CLAVE: Aguacate, gran-escala, portainjertos, combinación injerto-portainjerto.

DEVELOPMENT OF METHODS FOR AVOCADO ROOTSTOCK SELECTION IN ISRAEL

SUMMARY. During the present century, the avocado (Persea americana Mill.) has been developed into a commercial fruit crop in some countries including Israel. The practice is to graft cultivars on rootstocks. The trees have been found to be very sensitive to sorne soil stresses but different rootstocks vary in their sensitivity. Very little information has been available regarding the effect of rootstock on the development, resistance and productivity of the grafted tree. A large-scale field study appeared to be essential to collect the required information and to further successful development of the crop.

The present article is devoted to the techniques developed in the course of the large-scale project under which 700 field experiments have been set up since 1968, in commercial orchards. Seeded rootstocks were used in the first set of 350 experiments, involving 120,000 trees, and clonal rootstocks in the second set of 350 experiments, involving 60,000 trees. The experiments were set up in cooperation with the growers and followed a simple design. Follow-up surveys for about 10 years in each experiment covered stress

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symptoms; tree-size, measured from aerial photographs as ground canopy projection area; and yield, as weighed by the growers. Calculations and statistical analysis were done for cumulative yield, area occupied by the tree, tree efficiency" - in yield par unit area, and alternate bearing. Uniformity of each group of trees was calculated as CV. (coefficient of variance). Negative selection of rootstocks and sources of scion, based on the experimental findings led to improvement of the propagation material used, from the beginning of the research. At the end of first set of experiments, about 40 superior rootstocks were recommended for commercial use, and about 100 new clonal rootstocks were developed from outstanding trees. The latter were evaluated in the second set of experiments.

KEY WORDS: Avocado, Large-scale, Rootstocks, Stionic combination.

Development of methods for avocado rootstock selection in Israel

Sophisticated research methods mainly based on molecular biological principles, have became very popular and widely in biological and agricultural research institutions in recent years. However, these methods cannot replace field evaluations of the resulting new cultivars and agricultural techniques. With regard to fruit tress, the long-term evaluatíon of the development, disease and stress resistance, productivity and fruit quality of tress will need to be continued in experimental orchards, even if new cultivars and rootstocks shouid eventually be produced by modern technoiogies.

On the other hand, fíeld studies, especiaiiy with fruit rees, *are very* expensive and have to be continued for long time. In many cases the results are disappointing in both the researcher and the potential user of the developments, owing to the limited scale, and the small numbers of locations and replications. In some long-term experiments the results obtained in one location cannot be repeated elsewhere.

An example of a large-scale, long-term project, initiated after many years of research on rootstock breeding for apples and other deciduous fruit trees, was the comprehensive project called NC-140 (Ferree and Perry, 1988), in which about 50 experimental plots were established in parallel all over the apple belt of the J.S.A. and Canada. The two questions to be answered by such groups of experiments are: to which range of local conditions is any rootstock adapted? and what are the variation among rootstocks, relative to one another in their suitability for different locations?

The avocado (*Persea americana* Mill.), a tropical fruit from Central America was developed into a commercial crop in Israel during the 1950s. The avocado tree was found to be sensitive to several soil stress !actors, such as salinity, lime, poor aeration and diseases, and differences among rootstocks in resistance to these stresses were discovered. About 600 different rootstocks were in use in Israel at the end of the 60s but very little knowledge was available regarding their effect on tree development and productivity. Therefore, a large-scale, long-term field research study seemed to offer a very promising means to fill the gaps in the available data about the rootstock effects under differing growth conditions and for various cuitivars.

The newly established research system was described in the literature (Ben-Ya'acov, 1972, 1985) and experimental results have been published (Ben-Ya'acov, 1987).

The present article concerns the principles and methods that were applied in the research. We believe that they could be applied in other fruit studies involving questions shich are not readily answered either un modern laboratory research or in limited-scale field investigations.

Principles

The avocado rootstock case study was based on several principles which seem essential for such large-scale research:

1. The experiment should be established and managed in commercial orchards and the commercial orchards should be the research subject.

2. The growers responsible for the orchards, in which experiments took place, and their associated farm advisors were part of the research team and formed an active group called "The Avocado Rootstock Research Team' (Ben-Ya'acov, 1976).

3. The growers managed their orchards according to their own knowledge and experience and their preferred methods, and the central research team was responsible for the research activities.

4. The grower motivation to participate in the research was encouraged by his being the first to be informed about any data resulting from the experiment handied in his orchard; these data would be more applicable to his profit than to anyone else's, since they were directly reiated to his orchard. Also, more visits were arranged to the participating growers in order to ensure the follow-up of the experiments handled in their orchards.

5. The large scale of the experimental system is essential, in order to achieve reliable responses to many investigated treatments (rootstocks, sources of scion, agrotechnical methods), under a wide range of growth conditions. The idea that quantity can replace quantity can also be adopted, to a certain degree.

6. Owing to the large scale of the experimental system, only a small number of variables was studied, but these data (and calculations) were collected in each experiment.

7. The separate experiments and the whole system were designed according to satistical rules and can meet any scientific criticism.

8. The idea was to select a variety of rootstocks adapted to various cultivars, under different soil conditions rather than to look for one universal rootstock, which could never be found.

Research methods

In order to facilitate the establishment and management of avocado rootstock selection in a large-scale field research project, special methods were used; they are presented under the following headings:

I. Methods used for the establishment of the separate experiments and the whole system.

II. Methods used for the follow-up of the experiments.

III. Methods used for analyzing results and drawing conclusions.

IV. The mode of application.

1. Methods used for the establishment of the separate experiments and the research system

Many studies in fruit culture are characterized by their long-term nature. For this reason, proper design is highly important. In the research system presented here, about 350 long-term experiments dealing with seedling rootstocks were established during 1968-1978 and 350 experiments dealing with clonal rootstocks during 1979-1988. In the first part, 400 seeded rootstocks and 400 sources of scion were investigated and 120,000 trees were used in the experiments. In the second part, about 100 new clonal rootstocks were included and about 60,000 trees were used.

To enable the establishment of this Herculean task, the following steps were followed.

*A questionnaire was distributed among avocado growers asking about their planting plans.

*A survey was done in the avocado nurseries un order to identify the available propagation material.

*New sowings of avocado seeds and grafting of cultivars were directed and the followed up.

*Saplings were prepared in comparable groups of rootstock x scion combinations by grafting one source of scion (of a given cultivar) on different rootstocks (rootstock comparison) and different sources of scion on one (or two) common rootstocks (scion comparison).

*Toward the planting season, each year, a final survey of the nurseries was made in order to check their reliability and to ascertain what was the total inventory ready for planting. This annual survey also supplied a lot of data about the propagative characteristics of the rootstocks (Ben-Ya'acov and Sela, 1975).

*Planting maps, in which rows, columns, planting sites and cultivars were recorded, were prepared by the growers.

*Final maps, in which experimental blocks and rootstock x scion combinations on each site were recorded, were prepared by the central research team.

*In order to prepare the final experiment map, the experimental design had to be formulated. This was based on the preliminary map, made by the growers, and the list of saplings counted in the nursery. Usually the experimental designs were planned according to the "randomized blocks" system. The following steps were necessary:

1. To determine the number of stionic combinations included in the experiment.

2. To determine the number and form of experimental blocks included in the experiment.

3. To determine the representation rate of each stionic combination in each block.

4. To determine the forms in which combinations were represented in the blocks (either singles, couples, triplets, etc.)

5. To determine the localization of each group of representative sapling in each block.

*Each plant was tagged ¡n the nursery with a colored strip, according to the group to which it belonged using the same color used for this group in the maps.

*The saplings were transported to the field as separate groups.

*The orchard was planted according to the map.

*A general check of the set-up was made and maps were corrected if necessary.

II. Methods used for the follow-up of the experiments

A comprehensive, detailed and exact follow-up of each experiment (i.e., ah of the 700 experiments included in the project), for a period of about 10 years formed the main evaluation process. The following procedures were involved:

1. Working maps were prepared for each plot. They included:

    a) Complete maps un which ah details were recorded in regard to cultivar, rootstock and scion.

    b) Identical maps to group "a" but with blank spaces for details to be recorded by the growers or by the central team.

    c) Later, after the experimental data has been computerized, different types of maps could be prepared, based on the computer output.

    d) Maps of cumulative yield were prepared either manuahly or by the computer and served as the basis for activities in the orchard, such as thinning or topworking of poorly producing tree, etc.

2. Updating of the maps and the experimental lists with regard to changes in the orchards, was done continuously; changes in the tree inventory could result from tree death, appearance of "Sun Blotch" viroid, topworking of the cultivar, etc.

3. Surveys of the tree conditions with regard to stress symptoms were carried out when necessary, they mainly concerned leaf burns caused by sahinity,and chlorisis caused by lime. A grading scale for each symptom was deveioped in which grade "0', represents "no damage" and increasing numbers represent increasing degrees of damage.

4. Measurement of tree dimension was considered of high importance, as "tree efficiency" could be caiculated by dividing the yield by tree size. Trunk measurements do not look relevant for such calculations and measurements of the volume of the irregularly shaped tree would be a very laborious task; we preferred to measure the ground area occupied by the tree. The ground is one of the main producing factors at the grower's disposal, from this measurement and by calculation of the yield per unit area, he can learn how efficiently he is using the land occupied by different individual trees. This method could be helpful also, in guiding the grower with regard to the thinning rate and spacing needed for new plantings, according to the expected sizes of the trees in each group, which includes rootstocks and scion from different sources.

To meassure the area occupied by the tree, we first drew a une on the ground exactly under its margins. The area inside the une was measured as previously described (Kadman *et al.,* 1976) and designated "the tree canopy projection area" In order to carry out the measurements more efficiently and to facilitate data collection from many thousands of trees, we photographed the orchards from the air every 2-3 years and measured the projection area on the negatives. We used Kodak Aerochrome infrared films, and found that in midsummer the shade, which is very limited in extend, could be easily distinguished from the tree.

In practice, we measured the size of the trees by laying the negative on the backlit screen of a "Microview" instrument. A series of calibrated rings drawn on a transparent plastic sheet was moved by hand along the enlarged picture of the tree row. For each tree, the ring, closest in size was chosen, to provide the reading. The chosen ring was the one for which the parts of the foliage outside it were equal to the foliage-free area inside it. lo check the reliability of the measurements results, we correlated them with actual measurements in the field (Kadman et *al.,* 1976); we found high correlation values (r = 0.9) but had to apply scale correction factor (0.9) in order to adjust the oversized reading on the instrument to the actual measurements in the orchard.

The rings diameter in mm was fed into the computer, which converted it by a simple calculation that included the scale correction factor, into the area occupied by the tree in m^2

We also tried to use different sensors and to use image analysis techniques (Edelbaum *et al.,* 1988), but in practice no advantage was obtained.

Aerial photographs were used on a limited scale to evaluate the condition of the trees, especially with respect to chlorosis and necrosis. The technique proved useful to control tree stands in the orchards and prints were ordered by the growers for their own use.

5. Yield registration: Yields were recorded annually for each of the many thousands of trees included in the system. This was a huge task, but was essential for successful execution of the research. The avocado growers, individuals and kibbutz teams were the people who did it. They are convinced that to keep the yield record for each tree was important not only for the success of this research, from which they would expect to benefit, but also for the improvement of their own orchards. The productivity data collected showed a very wide variability in production among trees planted in the same plot, even if they were from the same stionic combination of the same cultivar. The information collected enabled the growers to improve the orchard productiviry by eliminating non-productive trees in favor of their more productive neighboring trees.

Yield was measured in kg through several different methods: some growers weighed the crop of each tree, even un the case of a few selective pickings during the year; most of them counted the number of picking containers - buckets, bags or Efron (picking machine) bags; and some of them estimated the yield, but they also expressed the evaluation in kg.

Yield data were transferred annually to the research center by means of yield maps or computerized reports. In most cases. This process continued for 6-8 years in each experiment. lo our knowledge (Ben-Ya'acov, personal data), this period is sufficient to express productivity potential of the trees.

6. Computerization: A special computer program was developed by Mr. Yigal Yahalom at the Meigar, Granot software house, to support the follow-up of the kibbutz avocado orchards, with special reference to the experimental system. The growers became used to working through their terminals and, later, to using this and subsequently developed software on their PCs. This subject is not described here in detail.

From the experimental point of view, the process was started by inputting the basic data of each experiment, including the exact location of each tree (plot, row and column), its planting date, the allocation of each tree to a certain block and a certain experiment, and identification of its rootstock and scion 'by code numbers' and its origin (nursery). Orientation data were included in order to enable the computer to output real maps. Subsequently, the input of current data became very simple as only the tree location had to be entered.

From the updated computerized information an annual output was received for each experiment, in the form of a report in which the current year's yield and the cumulative yield were presented for each stionic combination. The last 10 years can be displayed as a table together with survey and tree-size data. Maps of accumulated yields were produced according to the growers and the central teams needs. If needed, the maps can include data on certain trees only, either very productive or very non-productive ones.

III. Methods used for analysing and drawing conclusions

Each of the 700 experiments included in the two sets of experiments was treated by analysis of variance after its termi.riation, with regard to each collected or calculated variable.

Cumulative yield: Yield data were collected every year, but analysis of variance was made only for the perennial cumulative yield.

Measurements of tree proiection area: Measurements were done every 2-3 years when aerial photographs were taken. There was a limited period when measurements could be done, after the trees were grown aboye a certain size and before they became too crowded. Analysis of variance was made for the last measurement in each experiment.

"Tree efficiency" was calculated by dividing the cumulative yield of each tree by its occupied area as measured on the last photograph taken during the experiment and was expressed as kg/m^2 ; this was then analyzed as well.

Alternate bearing of each tree was calculated according to a formula suggested by Dr. A. Genizi of the Department of Statistics in the Volcani Center. According to this formula, deviations of yields from the biennial mean values were summed twice: once when the first yield is taken as the first of a pair, and then when the 2nd yield is the first. A general average of deviations was calculated, and the alternate bearing was finally expressed as a percentage: 100% meant that the tree did not bear fruit at alh, every second year; while 0% meant that the tree bore exactly equal yields every year, throughout the period. Of course, these two extremes are theoretical values which never were found in practice.

The alternate bearing formula is:

$$A = \frac{2}{n-1}\sum_{i=2}^{n}\left|Y_i - Y_{i=1}\right| / \left(Y_i + Y_{i=1}\right)$$

Population distribution: Mean values were subjected to statistical analysis, but it is also important to know how uniform is each population of trees, and what type of distribution characterizes the individual tree.

With regard to uniformity, the coefficient of variance (CV.) was calculated for each group of trees (representing one stionic combination) in each experiment. The CV. expresses the standard deviation as a percentage, and therefore enables different populations to be compared.

A study of the distribution of tree population was important for the first set of experiments, as rootstocks were propagated by seeds. lwo forms of data presentation were used: the Frequency Bar Chart of the SAS package, which presents each population as a histogram for every one of the above-mentioned variables and the Univariate which highlights trees which do not conform with population in regard to the several variables, and are much better.

In the second set of experiments, which dealt with clonal rootstocks, the last demostrations were not included in the data analysis but the CV. was calculated for the uniformity evaluation. On the other hand, new

criteria were calculated as suggested by Prof. H. Talpas. They were:

Precocity: Precocity has a high economic value but it could not be indicated when one is working with cumulative yield; therefore 10% was added to the value of yield, picked in the first years.

Production in "off' years: Assuming that most of the orchards do not produce during 0ff years, the fruit value increases. Productivity on off years was calculated as having higher value.

A general calculation: Different groups of trees evaluated in experiment for their rootstock and scion grading, and individual trees evaluated as candidates for clonal duplication, were ah finally subjected to a general summing up comprising a combination of the following variables: cumulative yield, tree size (canopy projection area), percentage of alternate bearing, precocity and off-year productivity. The fruit tree breeder can select for better rootstocks and scions or choose candidates for clonal propagation by using the general calculation or only some of its component criteria.

The evaluation of the complete set of experiments: The analysis presented so far has been based on the results and conclusions of every one of the many experiments separately. We found a way to evaluate propagation material included in different experiments, by including a common stionic combination in different experiments planted in one year, but we did not find any means of statistical analysis of the whole system or even a major part of it. Therefore, the general conclusions are based on logical analysis only.

IV. The mode of selection and application of the research results

During the 26 years in which the present research has been and still is taking place, several waves of avocado plantings have occured, and the total planted area in Israel has increased about fivefold. The research is intended to supply practical information to support the new plantings which could not await the completion of the research. Therefore, some applications have already taken place during the research period:

1. Negative selection of rootstocks: Roostocks that exhibited negative characteristics during the development of the experimental orchards were negatively selected and their use was terminated. Such negative characteristics included: sensitivity to chlorosis, degeneration, Sun Blotch viroid infection, etc. During the years, as yield data became available, rootstocks were eliminated when they were found to influence productivity negatively, relative to other rootstocks included in the same experiments.

2. Positive selection of rootstocks: During the research period and after the first set of experiments were terminated, we managed to identify certain rootstock sas the best producers in some experiments; they were recommended for use under conditions like those prevailing in the place where they were selected.

3. A list of rootstocks authorized for use: The recommended rootstocks were included in a first published annually in accordance with the law and improved from year to year. In 1970 the list included 630 different seeded rootstocks; in 1987 there were only 40, all of which were recommended according to the research results.

4. The establishment of mother plantations for seed supply: When better and even superior rootstocks became available, mother plantations were founded and they became the authorized sources for seed supply. Currently, four orchards supply West-Indian seeds and one Mexican.

The propagation material used for the establishment of these orchards was indexed for Sun Blotch viroid and, as the sources are isolated, reinfection can be prevented. Additional reasons for permitting nurseries to use seeds from these orchards only, are the elimination of cross pollination by commercial cultivars and the better control of the material in the nurseries, as it can be followed from the origin of the seeds.

5. Negative selection of scion sources: Some graftwood sources were eliminated in the initial stages of the research, as they were found to be infected with Sun Blotch viroid and some later, when they were found to be less productive than others. Wide variation in productivity were discovered un the Fuerte cultivar but rarely in other cultivars. Hass sources which were their non-productive or more sensitive to a phenomenon known only as "the phenomenon" than other sources of Hass, were localized and eliminated from use.

6. Positive selection of scion sources: When positive results had been obtained from graftwood from certain sources, the recommendation was made to use graftwood from the best trees included in the experiment. This became a process of rejuvenation of sources and elimination of the use of trees situated in old, viroid-infected orchards.

7. A hist of graftwood sources authorized for use: A list of selected sources of graftwood was pubhished annuahiy, ¡n parallel to the hist of rootstocks. Changes in the list occurred, with the elimination of viroid-infected and non-productive trees, and the above-mentioned rejuvenation process.

8. The establishment of mother orchards for graftwood supply: A few graftwood orchards were established, but they did not become the only sources of material as in the case of seeds. Supply of graftwood from young commercial orchards was continued as well.

9. The selection of rootstocks for clonal propagation: Selection of outstanding trees was done in the first set of experiments (seedling rootstocks), in order to supply rootstock candidates for the second set (clonal rootstocks). The criteria mentioned above, among others, were applied at this selection stage. The whole process will be described elsewhere.

10. The application of the research results in new plantings: In order to enable immediate application of the research results, "The Center for Coordination of New Avocado Planting" was established. Growers and farm advisors would visit the "center which was situated near the research unit, and propagation material was adapted to their necessities according to their local conditions. The best propagation material for each plot was then localized in nurseries, as the man in charge in the "Center" Mr. A. Ribenfeld, was also the nursery specialist and could supply the needed information.

SUMMARY AND CONCLUSION

Special research methods were developed and applied to a large-scale long-term field study dealing with avocado rootstock selection. In the present article, the methods have been described un detail in order to facilitate their use in research projects in which use of large-scale sets of experiments in necessary to solve problems that could never be solved by molecular biology methods, or by means of a single trial in an experimental station.

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