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ANATOMICAL AND MORPHOLOGICAL CHARACTERIZATION OF ROOTS OF SEEDLING AND CLONAL AVOCADO (*Persea americana* Mill) ROOTSTOCKS

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Anatomical and morphological characteristics of roots of clonal and seed avocado (*Persea americana* Mill) rootstocks were studied on nursery plants. For this research, 10-month-old ungrafted seedling rootstocks of Mexícola, Nabal, Reed and Zutano varieties and 18-month-old clonal avocado rootstocks of Duke 7 and Toro canyon varieties were used. Trunk diameter, leaf and root area, fresh and dry weights in both leaf and root were determined for each rootstock. Additionally, root morphology was assessed by identifying the type of roots (fine roots with only primary growth or thick roots with secondary growth) and the root branching order for each rootstock. The different components histologically examined were anatomically assessed, while the proportions of stele and cortex were determined. Clear morphological differences could be established between adventitious root system shown by clonal rootstocks and root system formed by a primary root and its ramifications presented by seedling rootstocks. Histologically in both clonal and seedling rootstocks, roots below 2 mm in diameter had a pattern of development different to that of roots with larger diameter, verifying a clear specification of the roots according to their diameter and rate of lignification. Finer roots with thicker cortex may function primarily for water absorption, whereas thicker roots with larger stele presumably function for water transport.

CARACTERIZACIÓN MORFOLÓGICA Y ANATÓMICA DE RAÍCES DE PORTAINJERTOS DE SEMILLA Y CLONALES DE PALTO (*Persea americana* Mill).

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Las características morfológicas y anatómicas del sistema radical de portainjertos clonales y de semilla de palto (*Persea americana* Mill) fueron estudiadas a nivel de plantas de vivero. Para esta investigación fueron utilizados portainjertos de semilla sin injertar de 10 meses de desarrollo de las variedades Mexícola, Nabal, Reed y Zutano y portainjertos clonales de dieciocho meses, de las variedades Duke 7 y Toro Canyon. Las plantas fueron evaluadas en términos del diámetro de tronco, área, peso fresco y seco, a nivel foliar y radical. También fue evaluada la morfología del sistema radical identificando los tipos de raíces que componen el sistema (raíces finas con crecimiento primario y raíces gruesas con crecimiento secundario) y la secuencia con la que los distintos elementos se presentaron. Anatómicamente fueron determinados los distintos componentes observados a nivel histológico y fueron cuantificadas las proporciones del cilindro

vascular y del cortex. Se pudieron determinar claras diferencias morfológicas entre el sistema radical adventicio presentado por los portainjertos clonales y el sistema radicular constituido por una raíz primaria y sus ramificaciones, presentado por los portainjertos de semilla. Histológicamente en ambos tipos de portainjertos raíces de diámetro menores a 2 mm presentaron un patrón de desarrollo distinto al de raíces de diámetros superiores, verificándose una clara especialización de las raíces de acuerdo a su diámetro y grado de lignificación. Las raíces finas y con mayor proporción de cortex estarían más relacionadas con la absorción, mientras que las más gruesas, con mayor proporción de estela estarían más relacionadas con el transporte.
Palabras clave: anatomía, raíces, palto, portainjertos, histología.

INTRODUCTION

The root system is described as not very deep and superficially extended in the area of projection of the canopy (Whiley *et al*, 2002). In spite of its importance in the absorption of nutrients, water and the synthesis of important growth regulators, it is an organ that has not been studied enough in this species.

Morphology studies the shape showed by the organs and their development, whereas anatomy studies the structure. In the case of the root system, the study of the shape and functioning of each of their components could reveal the implications of this organ in the storage, anchorage , acquisition and conveyance of water (Essau, 1979). Anatomical researches made on apple trees by Breakbane and Thompson (1939), on Kiwi by Wang *et al*., (1994) and on citrus fruits by Eissenstat and Achor (1999), show the strong correlation between the morpho-anatomical characteristics of roots and physiology of the plant. The present study shows a morpho-anatomical characterization of roots of different avocado rootstocks (clonal and seedling) with potential use in Chile.

MATERIALS AND METHODS

The first trial corresponded to the study of clonal rootstocks Duke 7 and Toro Canyon, plant material of great use as rootstock in the United States. This material is currently in Chile, but its evaluation on field has been recently implemented as well as its commercial propagation (FONDEF Project D04I1346-PUCV). Because of this, part of this trial was conducted in the US, in Brokaw Nursery, LLC.

During June 2006, rootstocks of each variety of the same characteristics in terms of vigour (foliage area and trunk diameter) were selected and evaluated regarding their morphology and root anatomy. To do this, the roots were washed first, taking care of losing the smallest quantity, their shape was photographed, and subsequently the types of roots found in each plant were selected based on their diameter and degree of lignification or colour.
Once the types of roots in the root systems from the different clonal rootstocks were identified, these were dissected and kept in FAA solution (10 formalin: 5

glacial acetic acid: 50 ethanol). Under these conditions, the samples were entered into Chile (prior inspection by SAG at the airport), and then they were analysed at the Histology Laboratory of the Biology Institute, PUCV.

The protocol for preparation of samples to be observed under microscope consisted in making progressive alcohol dehydration (50º, 70º, 95º and 100º for 30 minutes each). Then, a paraffin embedding was applied and through a microtome, the blocks were engraved in sheets of 14 µm. The dyeing of the samples was made with safranin and then they were tagged with *Fast Green*.

The samples were observed using an optical microscope (Olympus™ model BX40) equipped with a camera (Sony™ CCD-Iris model DXC-107A) and a camera adapter (Sony™ CMA-D2). The signal of the adapter was entered into a personal computer through a video capture card and WinTv™ software (Hauppauge Computer Works).

From each histological preparation, photographs were taken with a 10x target at a resolution of 640 x 480 pixels and the image analysis was made with Scion Image™ Beta 4.02 software (Scion Corporation). Through the present software, the area occupied by the stele and cortex was determined. The stele is the vascular cylinder located in the centre of the root and where the xylem and phloem are found. The cortex is the area of parenchymatic tissue that surrounds the stele and is limited in its internal part (in contact with the stele) by the endodermis and in its external part by the epidermis.

The research of the plant material propagated by the seed was carried out at the Propagation Laboratory, PUCV. In May 2006, seeds from 4 rootstocks commercially used in Chile were planted: Mexicola, Nabal, Zutano and Reed. These rootstocks were grown in cold greenhouse and once these reached an average of 10 mm in diameter at 10 cm high from the substrate (December), 6 plants were selected from each rootstock; the substrate of these plants was eliminated to evaluate the histological morphology and anatomy of the root system. Just like with clonal rootstocks, the roots were washed first, taking care of losing the smallest quantity, their shape was photographed, and subsequently the types of roots found in each plant were selected based on their diameter and degree of lignification or colour. The preparation of samples and methodology to observe histological sections is the same as that described for the clonal rootstocks.

RESULTS AND DISCUSSION

Morphological characterisation of the root system of clonal and seedling avocado rootstocks

In the root systems of clonal rootstocks, a root system composed by main adventitious roots can be observed in an average number of 10-15 and with an average 4 mm in diameter. Then, these ramify in secondary branches of approximately 2 mm in diameter, which at the same time ramify in tertiary roots of an average of 1 mm in diameter (Figure 1). The lignification in this type of roots grows as they become thicker. However, it is worth mentioning that in the Toro Canyon rootstock, a different category of 5 mm roots is detected (average diameter greater than the primary adventitious roots) and in addition to be thick, they are white (non-lignified); they also tend to arise directly from primary adventitious roots. They usually grow in the external part of the root system (area in contact with the bag) and have a great extension (Figure 2).

Regarding the average areas of the root systems (Table 1), it may be observed that although the total areas of the different rootstocks do not show great differences, these are found regarding the percentage distribution of the various root categories. In both rootstocks, the highest percentage of the total area of roots is occupied by secondary and tertiary roots (below 2 mm in diameter). In Toro Canyon, a different type of roots may be noticed (thick roots of great growth in length). In addition, a smaller proportion of adventitious roots can be observed. As previously mentioned, these thick roots would be an in-depth extension of adventitious roots; therefore, if they are considered as part of this area, the proportions of adventitious roots of both rootstocks would be also similar.

Figure 1: Root system and distribution of roots present in clonal avocado rootstocks. a and b: clonal Duke 7 rootstock; c and d: clonal Toro Canyon rootstock


Figure 2: Thick roots of clonal avocado rootstock of Toro Canyon avocado tree.

Table 1: Total areas and distribution of roots in the root systems of clonal avocado rootstocks

Rootstock	Total area (cm^2)	Area of adventitious roots (cm^2)	% of total area occupied by adventitious roots	Area of secondary and tertiary roots (cm^2)	% of total area occupied by secondary and tertiary roots	Area of thick roots (cm^2)	% of total area occupied by thick roots
Duke 7	652.05	203.42	31	448.63	69	0	0
Toro Canyon	695.26	104.28	15	521.44	75	69.54	10

The roots system of the rootstocks propagated by seed generally presents a long main root of thickness between 4 and 5 mm in the middle zone, very lignified and that ramifies in secondary roots of a mean 2 mm thick with a white or light brown colour (non-lignified). From these roots, tertiary roots of smaller diameter (1 mm), white and short (Figure 3) appear. It is worth mentioning that in the case of Reed rootstock, the presence of a curvature on the main root (10 cm deep), as well as a bifurcation in two of the root, could be noticed in a high percentage of the analysed plants (83%). Table 2 shows the areas of the root systems of the seedling rootstocks under evaluation.

Table 2: Total areas and distribution of roots in the root systems of seedling rootstocks

Rootstock	Total area (cm^2)	Area of main root (cm^2)	% of total area occupied by main root	Area of secondary roots (cm^2)	% of total area occupied by secondary roots	Area of tertiary roots (cm^2)	% of total area occupied by tertiary roots
Mexicola	270.76	66.08	24.3	83.72	31.0	120.96	44.7
Nabal	316.07	63.85	20.2	106.37	33.6	145.85	46.2
Reed	303.85	58.72	19.3	115.53	38.0	129.60	42.7
Zutano	268.51	52.17	19.4	113.14	42.1	103.20	38.5

* Equal letters in the same column indicate there are no statistical differences of significance of 0.05.

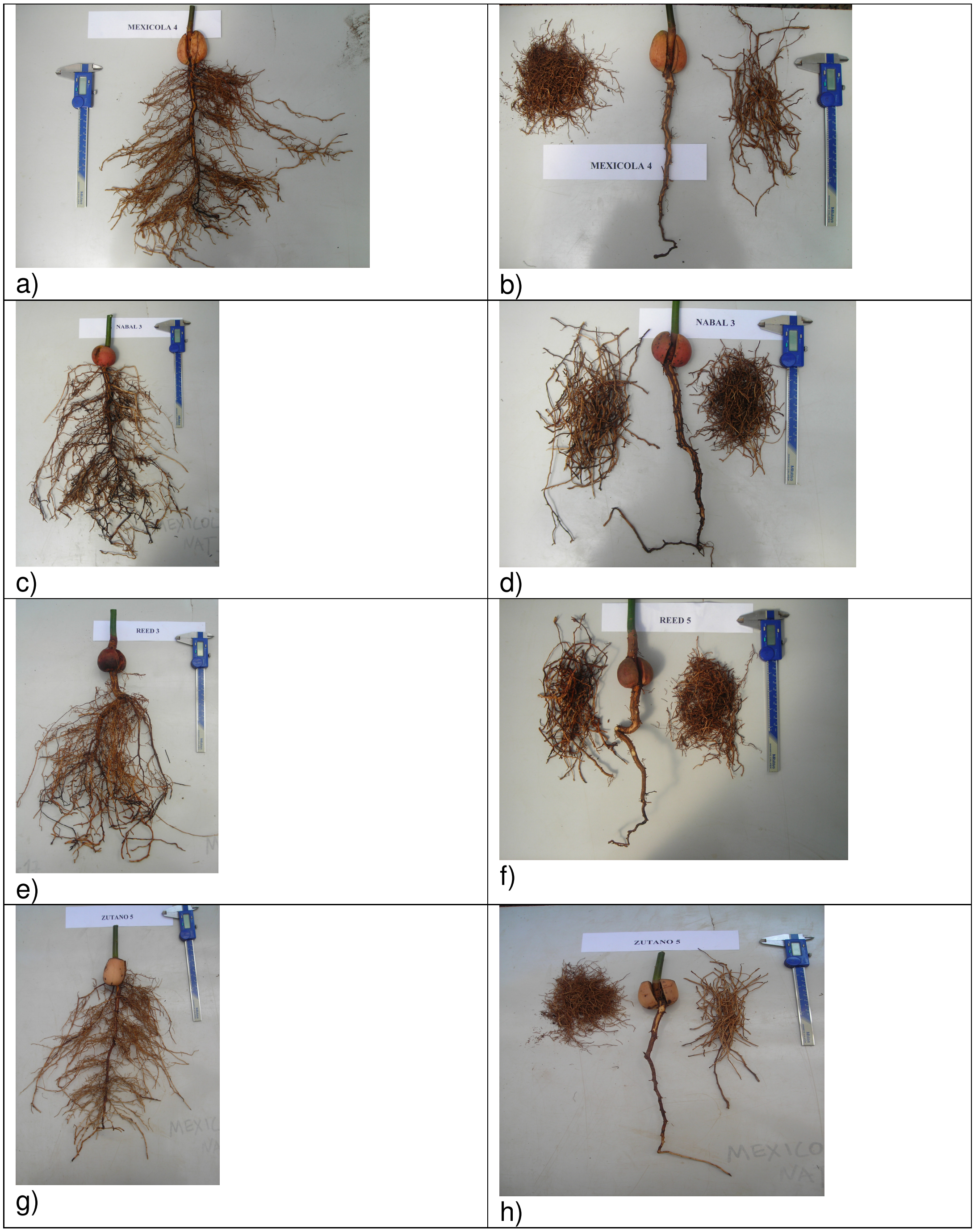

Figure 3: Root system and distribution of different types present in seedling avocado rootstocks. a and b : Mexicola, c and d: Nabal, e and f: Reed; g and h: Zutano.

The total areas of the root systems of seedling rootstocks in general do not show great variations among them. However, a difference may be noticed between the rootstocks of Mexican strain (Mexicola and Zutano) and those of the Guatemalan strain (Nabal and Reed). The latter show a root area larger than those of the

Mexican strain. It is worth mentioning that the seeds of all the rootstocks were planted at the same time, on the same substrate and kept under equal conditions; therefore, the differences detected can only be attributed to genetic aspects. Regarding the percentage distribution of the different types of roots among the evaluated rootstocks, it can be determined that in general the lowest percentage (around 20%) corresponds to the main root; this percentage is followed by those of the secondary roots (36%) and then by the tertiary roots (44%).

Anatomical characterisation of the root system of clonal and seedling avocado rootstocks

Anatomically, the avocado roots observed (of clonal and seedling rootstocks) usually present a cortex formed by parenchymatic cells. As the root diameter increases and the secondary growth in the cortex is observed, it becomes proportionally less important in relation to the stele. Contrarily to this, the stele, during the primary growth of the root, appears as an area of smaller proportion in comparison with the total area of the root. In the stele, the initial development of vascular cylinder composed by protoxylem and protophloem may be observed. According to the number of protoxylem poles, the roots observed in general showed pentarch, hexarch, heptarch, and oxarch patterns in their steles. As the roots increase in diameter, the proportion of the stele becomes more important. During the secondary growth of the roots, a complete development of the vascular tissue and the formation of the periderm in the external layers of the pericycle are observed. The cortex is completely displaced and its proportion regarding the total area is minimal. Lignification and increasing of the epidermis is also clearly visible. In Figures 5 and 6, the differences regarding the presence and proportion of tissues can be clearly noticed in roots under primary and secondary growth. In every rootstock, the roots with diameters below 2 mm presented a greater percentage of its area occupied by the cortex (over 85%). In contrast, this behaviour is reversed in roots with diameter above 4 mm, with the stele occupying the highest percentage of root area (over 65%). This could be caused by the level of root development, where roots smaller than 2 mm would be in a primary growth stage, and those of larger diameter in a secondary growth stage. This coincides with the functioning that the different types of roots should have, where roots of smaller diameter are the last ramifications of the root system, which is the area where the water and nutrient absorption mainly occurs. In contrast, roots of larger diameter and with a higher level of development and lignification would be in charge of carrying these elements; therefore, they would have a greater development of the conductive tissue

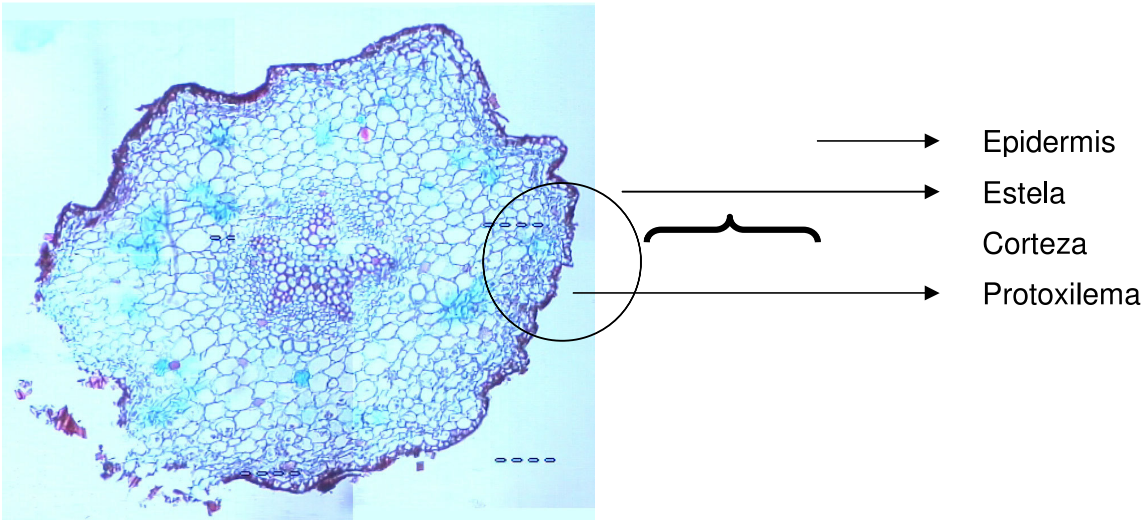

Figure 4: Primary growth in a pentarch root of 1 mm in diameter present in the clonal Duke 7 avocado rootstock (10X)

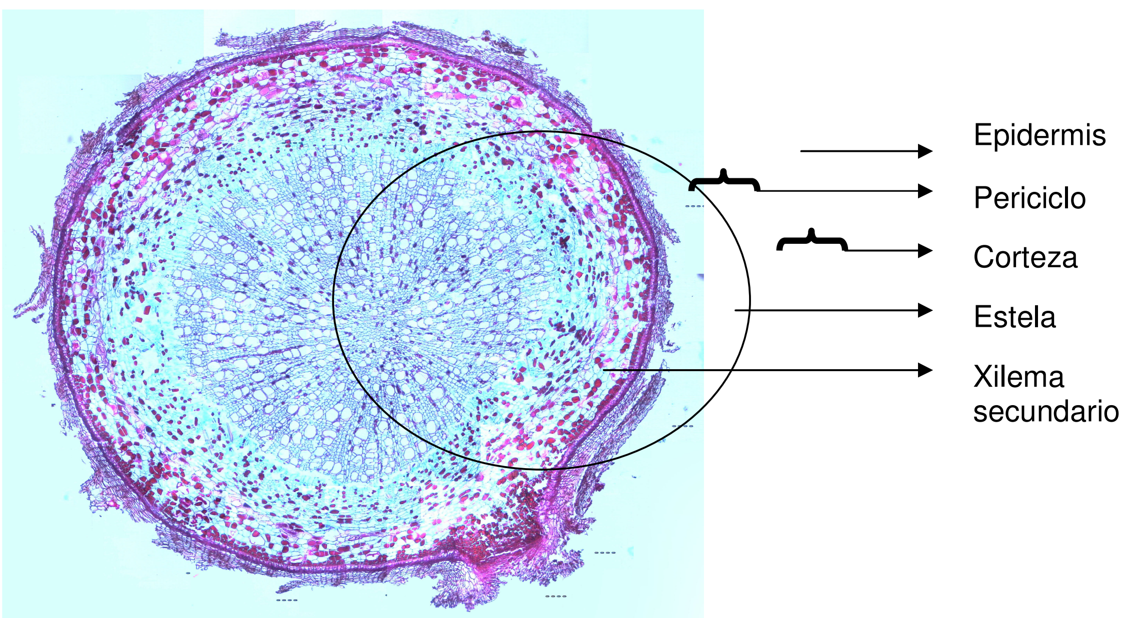

Figure 5: Secondary growth in a root of 4 mm in diameter present in the clonal Duke 7 avocado rootstock (10X)

The presence of roots with diameter above 5 mm in the Toro Canyon rootstock is significant, usually having only primary growth with a great proportion of the area occupied by the cortex (above 90%) (Figure 6). Because of the morphology shown by this type of roots (great growth in terms of length), these roots could have a different functioning, which would be related to the expansion of root growth towards the deeper areas. This would grant this rootstock a special characteristic regarding a greater capacity to explore new zones of growth in the soil. No description information is available on this type of roots (not many studies of the anatomy of avocado roots currently exist); therefore, continuing this field of investigation will be very important in order to determine the real functioning of this type of root.

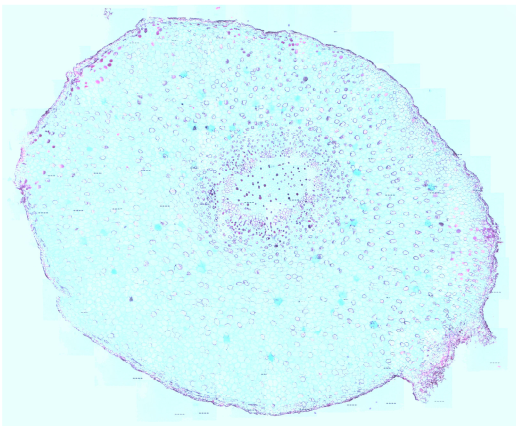

Figure 6: Anatomy of 5-mm roots present in clonal Toro Canyon rootstock (10X)

When statistically analysing the stele area (vascular cylinder) present in the studied rootstocks, it may be indicated that differences in this tissue (Table 3) are detected only in roots of 4 mm in diameter; where Duke 7 and Reed rootstocks show the largest area of stele and Mexicola the smallest one. The differences among the rootstocks at this point could indicate a greater capacity of water and nutrient transport in Duke 7 and Reed rootstocks.

Table 3: Stele area present in roots of different diameters in clonal and seedling rootstocks

ROOTSTOCK	Stele area (mm^2) in 1 mm roots diameter	Stele area (mm^2) in 2 mm roots diameter	Stele area (mm^2) in 4 mm roots diameter	Stele area (mm^2) in 5 mm roots diameter
Duke 7	0.066 a	0.55 a	6.66 c	-
Toro Canyon	0.059 a	0.48 a	5.87 bc	0.92
Mexicola	0.065 a	0.55 a	3.5 a	-
Nabal	0.072 a	0.41 a	5.3 abc	-
Reed	0.083 a	0.57 a	6.8 c	-
Zutano	0.088 a	0.38 a	3.2 ab	-

CONCLUSIONS

This study is very important for the knowledge of root architecture and functioning that the different types of roots present in the studied rootstocks could have, since no researches on this issue are currently available. The knowledge of these characteristics of the root system of nursery plants permits us to have a better approach when researching the rootstocks on field. The researches on other species prove that the architecture developed by nursery plants forms the base on which new growths on field occur. In addition, a strong correlation is detected between the anatomy of the roots produced by the different rootstocks and their functioning in terms of water and nutrient absorption.

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