

Genetic resources and nursery management



■ *Persea parvifolia* will. The possible ancestor of the avocado

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Persea parvifolia Will., was described by Louis O. Williams in 1977 and was not considered by Lucille E. Kopp in her 1966 taxonomic revision of *Persea*. A few years ago (2009) Francis G. Loera-Hernandez listed the species as a synonym for *Persea pallescens* (Mez) Loera-Hernandez mainly based on the type of pubescence on leaves and as L. O. Williams placed it under the subgenus *Persea*, that are also characterized by pistils with pubescence. *Persea parvifolia* is a species that is a shrub than a tree, described as a tree of small height, and this is due to their thin branches and small, narrow leaves. Its fruits are obovate to slightly pyriform, with very little flesh, with a length of about 3 cm and thin green skin. In our trials with grafts, it is vegetatively compatible with the three races of *Persea americana* Mill. and not with *Persea cinerascens* Blake, confirming its location in the subgenus *Persea*. In addition we clonally propagated this species without problem with the method etiolation. In studies conducted by one of our students María Edith Cruz-Maya in 2011 with the help of eight DNA sequences of chloroplast, mitochondria and nucleus, that this species has the most conserved sequences among other member of the subgenus *Persea* and clearly distinguishes from the subgenus *Eriodaphne*. This evidence makes it one of the most likely candidates that originated the avocado.

■ Description of hybrid morphometric avocado (*Persea americana* Mill.)

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A growing avocados potential problem is the lack of alternative cultivars to 'Hass', which covers 95% of the cultivated area in Mexico; this marks the need for quality and similar cultivars or greater than 'Hass' genetically different, which assures us more stability in production areas productivity. Therefore the aim was to characterize a population of 106 trees of 'Hass' x 'Pionero', by using numerical taxonomy, to highlight the novelties of the individuals in obtaining new varieties. The variables that determined the differences between materials were firstly characteristics of pedicel, fruit and seed; second were inflorescence, fruit and pedicel; and thirdly inflorescence and leaf characteristics. Qualitative characteristics that explained the most variability in the population were undulation of margin of the leaf blade, presence of depression at stalk end of the fruit, adherence of skin to flesh, anise aroma of flesh, seed coat: adherence to flesh, seed coat: adherence to cotyledon. Quantitative characteristics that explained the most variability were fruit length, fruit diameter, ratio length / diameter fruit, fruit weight, length of pedicel, ratio fruit length / seed length, seed length, diameter seed and seed weight.

■ Innovation, technological development and transference of young clonal avocado trees in Chile

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After 10 years researching in Chile under different soil and climatic conditions and with several limitations, the rootstock research program developed by PUCV's Propagation Laboratory has shown that clonal avocado rootstocks are a real alternative against limiting conditions for this species. Because of the increasing age of the avocado orchards and their decay, the current use in Chile of these rootstocks mainly under replanting conditions, has increased exponentially, going from 571 avocado plants on clonal rootstocks marketed in 2008 to 70,000 clonal plants sold in 2015 (ODEPA, 2013 and clonal nursery men associated to PUCV's cloning program).

This commercial development has not been exempt from difficulties, since for achieving quality clonal plants, that is with a considerable amount of adventitious roots that could be evenly distributed, hard work is necessary mainly on the first development stages of this plant, which has been dubbed YOUNG CLONAL TREES. This stage suffers the largest percentage of lost plants, bordering 50% in the different stages, in addition to the high cost in infrastructure and energy for heating.

Commercially in Chile, 5 nurseries sell clonal plants but only 2 of them include the whole cloning process. Given technical and economic difficulties during the process, the remaining 3 have decided to work together with PUCV's Propagation Laboratory, specialized in growing young clonal trees because of the technological development they have reached and as a strategy for transferring this technology. This has increased the production and marketing of clonal plants, currently having an annual demand of around 100,000 plants.

Multi-location rootstock evaluation in Australia

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Field trials evaluating several parameters of growth, fruit yield and quality of “Hass” and “Shepard” avocado grafted to 22 seedling or clonal rootstocks were established in 2004-2005 in four different growing regions in Australia. Detailed multi-environment repeated measures data analyses are underway, for yield, yield efficiency and fruit size. Preliminary analysis shows that highest yielding rootstocks were from the West Indian and Guatemalan horticultural races, and Mexican x Guatemalan hybrids the second most successful group. There was no single rootstock that was superior across all production regions. Orchards in tropical and subtropical areas (Queensland) had higher yields than the orchard in temperate south-west Western Australia. Severity of Phytophthora root rot and postharvest fruit diseases were significantly different amongst rootstocks.

Avocado rootstock research: principals and practices

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The value of a reliable clonal rooting technique has been reported. A direct correlation between the quality of a rooting system and tree uniformity has been illustrated. Clonally propagated rootstocks is the only way to express certain outstanding tree characteristics of the scion, such as precocity and production. However, seedling rootstocks are predominantly still used in many countries as the preferred rootstock. The effect of temperature and growth medium to improve root quality and quantity as well as the influence of different rootstocks on the field performance of ‘Hass’ and ‘Maluma’, has been studied. Using the Allesbeste micro cloning technique with coco peat as growth medium and a rooting temperature of between 20 – 28°C ensures superior clonal root development. With ‘Hass’ as the fruiting cultivar, ‘Duke 7’ outperformed ‘Duke 7’-seedling rootstocks on production and uniformity, while on production ‘Duke 7’ and ‘Thomas’ outperformed ‘D9’ and ‘Barr- duke’; ‘Dusa’ (Merensky 2) and ‘Velvick’ outperformed ‘Duke 7’. Comparing horticultural characteristics of the scion, rootstock research with rootstocks grafted to ‘Hass’ has shown to merely improve certain inherent genetically limiting factors, while new generation cultivars have shown to maximise genetically existing attributes. Therefore Allesbeste Nursery decided to discontinue the use of ‘Hass’ and replace it with ‘Maluma’, as the standard for further rootstock evaluation. Under high density (808 trees/ha), the highest yield was recorded with ‘Dusa’, while ‘Bounty’ established best in a replant situation. An ultra-high density trial with 1600 trees/ha grafted on ‘Dusa’, ‘Duke 7’ and ‘Bounty’ is under evaluation. An extensive trial with 24 different clonal rootstocks, is to be planted soon.

Economic considerations of ‘maluma’

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The ‘Maluma’ cultivar has been in semi-commercial and commercial production for about 15 years, however in avocado cultivar commercialisation terms it is still considered quite new. ‘Maluma’ originated as a chance seedling of unknown parentage. For any new cultivar to be commercially viable it needs to present significant economic advantages in comparison to already existing commercial cultivars. A new cultivar should also mitigate some of the risks associated with the production and marketing of avocados.

Statistical data was obtained from within the South African context, while physical observations were also made in other countries where ‘Maluma’ has been planted commercially. Statistical data with regards to production and income per hectare as well as labour cost are analysed over consecutive seasons and compared to ‘Hass’ in the same environment for reference. The data was obtained from commercial orchards on different farms located in the main avocado production region in South Africa. Physical observations with regards to production and climatic factors are also analysed and compared.

Analysis of the data shows that ‘Maluma’ presents significant economic advantages in comparison with ‘Hass’ in terms of labour efficiency and income per hectare. It is shown that ‘Maluma’s’ economic advantages also lie in the fact that it mitigates certain risks relating to avocado production. These risks include factors such as climatic risk and variability of production. Statistical analysis indicates significantly less variability in seasonal production of ‘Maluma’ relative to ‘Hass’. Therefore it is concluded that ‘Maluma’s’ economic advantages establishes it not only as a viable commercial cultivar, but an attractive one for the entire value chain.

■ 'Maluma': establishing a new generation avocado cultivar commercially

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An industry dominated by a single cultivar, or that produces too few new cultivars, is vulnerable. Ironically no less than 13 fruiting cultivars presently receives intellectual property protection in various countries, of which less than half has established themselves commercially. Presently 'Hass' is the world's premium avocado cultivar, which originated as a chance seedling planted in 1925 by Rudolph Hass in La Habra Heights, California. A plant patent was granted in 1935 in California, which expired in 1952. 'Hass' only established itself during the 1970's, as the initial acceptance of 'Hass' by packers, distributors, retailers and end users, as a superior commercial cultivar was slow. 'Hass' received resistance because of its fruit being black-skinned, rough and small. 'Maluma', a new generation cultivar, was discovered, during the early 1990's, by Andries Joubert on his farm Maluma in Levubu, South Africa. Plant Breeder's Rights were granted to Allesbeste Nursery on 7 November 2004 and commercially released, after 8 years of intensive semi-commercial research, during November 2007 at the VI World Avocado Congress, Chile. Successful commercial establishment in the European Union and United Kingdom occurred within another 8 years. This achievement was made possible through the active integration of the following:

- Horticultural attributes
- Marketing attributes
- Competent, loyal participants
- Vibrant trade relationships
- Additional economic value for all participants
- Technical support, able to deal with the critics
- Branding and promotion
- Significant marketable volumes
- Strategic establishment to ensure future 12 months' market presence

This active integration was achieved through Allesbeste's vertical integration in all structures from propagation to exporting, providing a platform for efficiently applying its inherent skills in all fields.

■ The strategic role of new cultivars: a case study of 'Maluma'

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Cultivar choices are more difficult with new orchard development. Many new cultivars have been introduced during the last decade, each claiming its own unique benefits. 'Hass' continues to claim the premium avocado cultivar position in large parts of world, especially in the marketing paradigm. Furthermore farmers also need to adapt to the modern farming environment to remain relevant and efficient. After studying multiple commercial 'Maluma' orchards in Limpopo, South Africa, 'Maluma' was identified as a cultivar that is well suited for the modern farming environment. 'Maluma', is a less vigorous, precocious and productive 'Hass'-like cultivar. The fruit turns to an attractive dark purple-black once ripened. Furthermore, over the past years it has proven itself to be one of the best suited cultivars for ripening programmes. In the South African context 'Maluma' has filled an important gap in the farmers' production and marketing basket. Although it is similar enough to be acknowledged as 'Hass', it distinguishes itself sufficiently to expand the range with which the farmer competes in the market. It does not merely compete with 'Hass' to the detriment of the farmers' current value proposition. This study proves the key production advantages of 'Maluma' in the farming environment such as high yields, precocity, suitability for high-density, susceptibility to diseases and orchard cold damage and other influences on quality. Also key marketing parameters of avocado such as appearance, count size, timing, cold tolerance, shelf life and ripening is differentiated. Ultimately this study proves to the farmer that establishing new generation cultivars are worthwhile.

■ Microma regulation of avocado adventitious rooting for clonal rootstock propagation

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Grafting fruiting scions to clonally propagated rootstocks has been reported by industry to significantly increase yields and tree uniformity compared to grafting to seedling rootstocks. In addition, the technique is labour intensive, and in high labour-cost countries such as Australia, it can make clonal rootstock trees prohibitively expensive, especially given the global trend towards high density plantings. A vegetative propagation technique that results in low cost plants is required. However, the difficulty of adventitious root generation from avocado stem cuttings makes propagation without the use of a nurse seed a significant challenge. The Frolich and Platt (1965) double grafting protocol and derivatives thereof (e.g. Brokaw 1977 patent #4012866, Ernst 1999) is the standard practice for rootstock propagation and whilst industry reports an un-interrupted etiolation step is crucial for rooting phenotypes, it is not yet understood why this is so. To address this issue, we looked at the expression of miR160, a proven adventitious rooting promoter in *Arabidopsis thaliana* and miR167, a rooting inhibitor, in avocado scions grafted for root clonal propagation. We determined that etiolation induces a spatiotemporal accumulation of miR160 in basal stem tissue compared to apical tissue, a trend that was not identified in non-etiolated equivalent scions. A time course assay sampling throughout the etiolation and de-etiolation steps has been performed to determine when key molecular changes are occurring, the results for which will be discussed. Understanding the molecular regulation of adventitious rooting in avocado will allow informed decisions for improving clonal rootstock propagation.

■ A comparison of Hass avocado rootstocks. Observations on the first 7 years

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The experiment was established in Malaga (Spain) on shale soil, well drained, not calcareous or saline. Irrigation water was strongly calcareous but not saline. Two replicates were planted in 2008 and one in 2009. Within each replicate the design was at random with approximately 15 trees per rootstock. All rootstocks were grafted with Hass bud sticks from trees selected for high long term tree efficiency. The following rootstocks were included:

ROOTSTOCK	ORIGIN	REPRODUCTION METHOD
Topa Topa	California (USA)	Seed
Zutano	California (USA)	Seed
Velvick	Australia	Seed
Gallo 3	Islas Canarias (Spain)	Clonal
XV-2	Málaga (España)	Clonal
Dusa	Republic of South Africa	Clonal
Latas	Republic of South Africa	Clonal
Duke 7	California (USA)	Clonal
Toro Canyon	California (USA)	Clonal
Uzi	California (USA)	Clonal
Steddom	California (USA)	Clonal
Thomas	California (USA)	Clonal
VC 66	Israel	Clonal
Hass	California (USA)	Clonal

In the first crop (2011), Zutano, Toro Canyon, XV-2, Gallo 3 and VC 66 had significantly higher yields per tree than Latas, Thomas and Topa Topa. The others were intermediate. In the next four crops, 2012 – 2015, VC 66, Duke 7, Velvick, Toro Canyon, XV-2 and Latas had the highest mean yields, between 14.2 and 15.9 kg.tree⁻¹.year⁻¹. Topa Topa and Thomas had the lowest mean yields (10.2 and 10.4 kg.tree⁻¹.year⁻¹). Differences in mean fruit weight were proportionally smaller. Only Zutano and Velvick had mean fruit weights under 220 grams. The others had mean weights between 230 and 243 grams except XV-2 that had 253.

Duke 7, Latas and Uzi showed the highest increases in trunk cross sectional area between 2009 and 2013. Dusa, Gallo 3, Hass, Topa Topa, VC 66, Velvick and Zutano the lowest. Steddom, Toro Canyon, Thomas and XV-2 were in an intermediate position.

■ Photosynthetic behavior in *Persea americana* Mill. seedlings of 'Duke 7' e 'Toro Canyon'

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The world avocado production for exportation is increasing in the last decade and with this, the need for improve the quality seedlings production. Seedlings of *Persea americana* Mill, from open-pollinized seeds of 'Duke7' and 'Toro canyon', developed for six months under greenhouse conditions, were evaluated in order to determine the relative levels of patterns of chlorophyll index a, b and total and photosynthetic efficiency of photosystem II (Fv/Fm), as physiological indicators of stress conditions. The determination of the chlorophyll content index in the plant profile was measured from the second to the fourteenth fully expanded leaves; Fluorescence measurement was performed in the third, fifth, seventh and ninth fully expanded leaf, and determined its variation in a function of time and intensity of the exposure light source, and the time for pre-adjustment of the dark leaf; was also compared with the Fv / Fm between the right and left sides of the leaves. The chlorophyll contents index were not different between left and right side but was different between tested materials, being observed in the 'Duke 7' the largest value from ninth leaf, while among leaves of "canyon Toro was not different. Similarly, for the Fv / Fm there was interaction of the three factors in the two materials, being the major exposure time (9 seconds), as well as little time to adapt to the dark and low intensity exposure did not induce maximum levels of fluorescence. On both materials, exposure to 100% of the light intensity for 7 seconds, prior to dark adaptation between 25 and 30 minutes was the most adequate combination for the measurement of photosynthetic efficiency.

■ Factors affecting avocado shoot health in culture

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Avocado tissue culture systems have the potential to provide a sterile, high-throughput, cost efficient alternative for clonal avocado propagation. Tissue-culture potentially allows multiplication of clonal stocks to provide much more cost efficient, uniform, reliably high-yielding plants that are guaranteed disease-free. However, clonal propagation of avocado in culture is complicated by high inter-cultivar variability in explant health in culture.

We are investigating avocado culture media for shooting of nodal explants from mature trees of a number of varieties. Numerous variables were found to affect shoot growth and survivability, and the effect of carbohydrates in the media will be discussed here. We are also examining endogenous carbohydrate profiles in explants harvested for tissue culture. The effect of etiolation on these profiles, and also on the carbohydrate profiles in budwood grafted for clonal propagation using the traditional Brokaw method (patent #4012866, Ernst 1999) are being determined. Carbohydrates are essential for energy and developmental signalling in plants and we aim to relate our results to plant growth rates and propagation.

■ Gibberellin and Cytokinin in synergy for a rapid nodal multiplication system of avocado

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Conventional clonal avocado rootstock propagation relies on a 40 year old method and derivatives thereof that is labour intensive and does not facilitate cost effective supply of clonal rootstocks for the rapidly developing needs of the industry. This is especially relevant if high density plantings or clonal rootstocks are to be adopted. An alternative tissue-culture-based clonal propagation system could provide efficient, cost effective, high volume and sterile production of clonal rootstocks. Avocado, being a woody plant species, is not highly amenable to tissue culture, with most attempts confined to nodal culture methods. Thus far, nodal cultures of avocado show very slow growth and produce stunted shoots, limiting rapid shoot multiplication.

The current study aims to develop a system to multiply nodal avocado shoots using gibberellins and cytokinins to produce taller shoots with a higher number of nodes for continuous culturing. Cytokinins, the most important plant growth regulator in shoot regeneration, and gibberellins, which promote internode elongation, can synergistically improve the quality shoots for rapid nodal multiplication. The effect of different combinations of the natural aromatic cytokinin, metatopolin, and the active gibberellin, GA3, on bud breaking time, shoot height, number of open leaves, overall shoot quality, vitrification and callus production in continuous culture of avocado nodes was assessed. The results reveal optimal hormone combinations for improved nodal multiplication for the avocado tissue culture pipeline.

■ Presence and development of the Hass Carmen Mendez in Mexico

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From first grafts of the variety and for the big commercial advantages that are achieved, this selection continues experiencing a spectacular growth both in Mexico and in other producing countries, the intention of this presentation is of showing of the way as objective possible the development that giving this in Michoacán's condition and Straw hat and the importance that it represents for the producers and exporters to rely on this alternative of production that it allows to have supply of avocados of good quality and content of oil in the months in which only one was possessing the sporadic possibility of having some production in the very benign years of mad fruits, Allowing to the industry to possess a supply as reliably to cover the demand of product during this period, both for the export and for the domestic market,

Current state of the avocado Hass Carmen Mendez in Mexico.

In the last years one has experimented on Mexico and on other producing countries of avocado, a notable increase on the surface cultivated with the variety Carmen Méndez. The precociousness of his production is turned by them into an excellent alternative for the supply of the domestic markets and of export into the months of the year into that Hass's offer is low. This presentation focuses in emphasizing the competitive advantages of the variety and his evolution in Mexico, With the intention of stimulating his adoption in the industry aguacatera world.

■ The Israeli avocado breeding program - past, present and future perspectives

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Avocado is an economically important crop worldwide. In Israel, the current extent of avocado plantations is 7,000 hectares and they are expected to yield more than 115,000 tons of fruit. The avocado harvest season in Israel starts with cv. 'Galil', followed by 'Ettinger', 'Hass', 'Fuerte' and 'Reed', which is picked last. Despite their popularity, our commercial cultivars possess some defects including high alternate bearing index and small size fruit, especially in 'Hass', a short harvesting season in 'Ettinger', and low fruit quality in 'Fuerte', thus creating a need for increased cultivar diversity. The Israeli avocado breeding program started in the early seventies and lately was renewed. Currently, our breeding project objectives are to select new 'Hass-like' avocado cultivars superior to 'Hass' in productivity and fruit size and to select early and late season green skin new cultivars, suitable for local climatic conditions. Our breeding strategy is based on both open pollination and controlled crosses using diverse cultivars of our germplasm collection. The controlled crosses are performed with caged trees with bees as a pollination vector. During the last decade a number of new cultivars were selected including the 'Hass' – like new cultivars: 'Naor', 'Lavi' and 'Bar' and the green – skin 'Moti'. These are being registered in Israel and in additional avocado growing countries. A summary of past and present avocado breeding program achievements, as well as future perspectives will be presented.

■ Evaluation of avocado accessions seeds donors to stress conditions: irrigation with saline water and alkaline soil

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World demand for avocados is pushing to establish groves on marginal soils and use of irrigation water whose quality is questionable or unsatisfactory for avocado trees. High soil pH and total concentration of soluble salts in water are the limiting factors. Then rootstocks propagated using seed material collected from trees identified by the avocado network and nurserymen of Mexico relative to higher seed germination percentage, vigor, health and uniformity of seedlings, were tested in two experiments under greenhouse conditions. In one experiment 135 seedlings of 9 accessions var. drymifolia and americana from *Persea americana* and *Persea nubigena* were irrigated with water having an electrical conductivity of 3 dS m⁻¹ in a completely randomized experimental design. In a second experiment, 210 seedlings of 10 accessions from the same varieties, were grown in soils of different pH: 6.4, 7.6 and 8.5, using the same experimental design. The Navideño and Hunucma accessions both of americana var. grew more vigors (Navideño had increases in plant height and stem diameter superior to 40%) and had a lower necrosis intensity on leaves due to excessive concentration of Cl⁻ and Na⁺. This response was associated with the distribution of Na⁺ in the root and stem, relative to Cl⁻ which was concentrated in the root. In alkaline soils, accessions Navideño, Reyes y Aurelio from var. americana and Tochmilco S2, Aquila S1 and La Joya from var. drymifolia showed a higher tolerance to iron chlorosis associated to higher root density.

■ Responses of newly developed south african 'fuerte-type' avocado selections to postharvest storage temperature and shelf-life

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The number of preferred South African avocado cultivars exported to European countries is limited to only four major cultivars. However, to remain competitive the industry must continue to breed superior new cultivars with high paying export price. To mitigate these challenges, the Agricultural Research Council-Institute of Tropical and Subtropical Crops (ARC-ITSC) has developed new 'Fuerte-type' avocado selections. For these selections to be registered they must be evaluated at a mandatory low temperature storage. The objective the study was to evaluate response of new 'Fuerte-type' avocado selections to industry recommended low temperature storage simulating export conditions and shelf-life. Mature fruits were randomly harvested from a gene block at ARC-ITSC Burgershall research farm in Hazyview. At the laboratory, fruits were stored at 5.5°C for up to 28 days. During ripening fruits were evaluated for electrolyte leakage, weight loss, firmness, colour change, carbon dioxide production, physiological disorders and postharvest diseases. The four evaluated selections (ITSC selection, Calshad, BL1058 and Wurtz) maintained colour parameters, which was consistent with commercial Fuerte cultivar. After cold storage, Calshad and ITSC selection showed lower fruit weight loss, respiration rate, electrolyte leakage, and ripening percentage which correlated with no external chilling symptoms. An increase in weight loss, respiration rate, ripening percentage, electrolyte leakage was observed in Wurtz and BL1058. Results showed that Wurtz and BL1058 were not susceptible to external chilling symptoms at the mandatory cold storage temperature and had a longer shelf-life. Therefore, the two selections would be recommended for registration.

■ The response of 'Hass' avocado to salinity as influenced by rootstock

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With increasing water demands, drought, and extreme high or low temperature environment worldwide, avocado growers are challenged with reduced water availability and lower water quality. Salinity is a critical issue for California avocado growers and, coupled with avocado root rot, threatens the long term sustainability of the industry since avocados are known to be extremely sensitive to high soil electrical conductivity (EC). Salt tolerance of 'Hass' is influenced by rootstock. We investigated 13 Phytophthora root rot tolerant clonal rootstocks grafted with 'Hass' from California and South Africa under high salinity (1.5 mS EC with 175 ppm Chloride) from 2013 to 2015. Results showed a range of tolerance to salinity due to rootstock. There were no significant differences in the number of fruit between treated and non-treated trees after treatment of 3 months; however, there were significant differences among the rootstock varieties ($p=0.0006$). There were significant differences in the number of fruit, between treated and non-treated trees after treatment of 13 months ($P=0.0173$), and fruit dry weight in Dusa, PP40, and R0.05 ($p=0.0352$). Physiologically, there were significant differences in leaf damage of 'Hass' attributable to rootstock due to the salt treatment but no differences between rootstocks under the fresh water treatment. There were significant differences between photosynthesis/transpiration and intercellular CO₂ rates within the saline treatment but not the fresh water treatment in tested rootstocks, Dusa, PP24, PP4, and R0.05 ($p=0.0007$, $p=0.0018$). These are indicative of different levels of water use efficiency in response to salinity. Studies into the responses to saline treatment at the gene expression level are in the progress and will also be reported.

■ 30 Years of cultivar research at Westfalia technological services

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The search for superior avocado cultivars by Westfalia Fruit Estates was initiated ca. 30 years ago, with a formal cultivar evaluation programme being introduced in the early 1990's. The aim of this programme was to find high yielding, good quality and robust cultivars to compliment the traditional cultivar spread, both on a local and a global level. Over the years Westfalia Technological Services (WTS) have sourced and tested over 150 cultivars grown either in South Africa or from other avocado producing countries where they either originated from a formal breeding program or were discovered as chance selections. Only a small percentage of these cultivars have been developed to a commercial level due to the strict quality, seasonality and production criteria imposed on them.

The evaluation programme consists of a series of rigorous screening phases that need to be completed before a cultivar will be released for commercial development. An example of the success of this programme has been the introduction and commercialisation of two new avocado cultivars in the last few years that have enabled Westfalia Fruit to offer their high-end clients (locally and internationally) superior quality fruit for twelve months of the year. The screening phases, and lessons learnt will be discussed in more detail in this paper, as well as the importance/significance of collaborating with avocado breeders from around the world.

■ Somatic embryo cryopreservation of multiple avocado (*Persea americana* Mill.) cultivars using two vitrification based techniques

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Avocado genetic resources are currently maintained in the form of field repositories at great cost and risk of natural disasters, pest and diseases. Cryopreservation offers a necessary, complimentary method that is safe, cost-effective and long-term. Long-term maintenance and regeneration of plantlets from avocado somatic embryos has been a major barrier in the development of new avocado varieties due to low germination rates and loss of embryogenic competence.

Somatic embryo viability to different sucrose concentrations in loading solutions, the effect of Plant Vitrification Solution-2 (PVS2) incubation times and temperatures were optimised. Two protocols for vitrification-based cryopreservation (cryo-vial and droplet) of avocado somatic embryos were investigated. Globular somatic embryos of three avocado cultivars (A10, Reed and Velvick) were tested, and the ability to withstand cryopreservation is attributed to cultivar-dependent differences in desiccation tolerance and subsequent freezing resistance, possibly due to their size and culture age. Somatic embryos were stored in liquid nitrogen for 3 and 12 months. Somatic embryos were successfully recovered post liquid nitrogen using a rapid thawing system at 37°C. Higher frequencies of regrowth of somatic embryos ranging from 75 -100% were obtained on MMSE after 5-10 weeks. Mature somatic embryos selected from MMSE plates were regenerated using a two-step regeneration system (Encina et al 2013). The system developed for storing and regenerating avocado somatic embryos for multiple cultivars will add considerable value towards cryopreservation of avocado germplasm as well as the generation of new and improved avocado varieties.

■ Optimizing conversion of transgenic avocado somatic embryos

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Use of biotechnological tools in avocado (*Persea americana* Mill.) is hampered by difficulties in obtaining mature somatic embryos with an acceptable germination capacity. Use of semi-permeable cellulose acetate membranes on top of maturation medium has improved the quality of obtained embryos and their germination rate (Palomo-Ríos et al. 2013); however, generally, the transgenic embryos, obtained through *Agrobacterium* inoculation, develop buds ≤ 2 mm, which fail to elongate. In these cases, cotyledons should be removed and the embryonic axis cultured over 4 weeks in MS medium supplemented with cytokinins (1 mg/l BA and 1 mg/l TDZ). Sprouting shoots could be either induced to proliferate in GD medium (Gamborg et al., 1968) supplemented with 0.3 mg/l BA, or grafted onto in vitro germinated seedlings (Pliego-Alfaro and Murashige, 1987). Following this procedure, 4 transgenic lines harbouring the GFP-GUS as markers, 2 lines harbouring DsRed and one with GFP were obtained. In addition, 4 transgenic lines with AtNPR1 gene could also be recovered. Interestingly, noticeable differences were found in morphogenetic capacity among transgenic lines harbouring the same construct. Financial support: Ministerio de Ciencia e Innovación, España, Grant AGL2011-30354-c02-01

■ Morphological and molecular characterization of avocado germplasm in the center of Mexico

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The center of Mexico is a region where diversity of native avocados grows, some of them used by people and other without previous knowing prevails. For years the ex situ conservation of some genotypes has been carried out, but the main conservation work is developed by the villagers so *in situ*. Knowledge of the diversity of the genotypes in the region comprises description and identification job, meanwhile their attributes are known: highlighting the potential use as a variety for fresh consumption, industry, rootstock or attractive ornamental. The objective of this study is to develop a morphological and molecular description of individuals to know the diversity and variability in the region and distinguish their similarities. In a sample of 45 individuals based on morphological characteristics and DNA polymorphisms, have been distinguished related species as *Persea cinerascens*, *P. floccosa*, and the variability of *P. americana* var *drymifolia*. Groups were compared with *P. shiedeana*, *P. nubigena*, *P. steyermarkii* and some relationships are discussed according to the generated dendrograms. The identification of genotypes, their location, efficiency of the morphological and molecular markers is showed and discussed.

■ New clonal rootstocks are showing their value

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In the last 15 years NZ Avocado has developed a programme for new cultivars which has focused in the importation and evaluation of Hass-like fruiting cultivars and tree decline tolerant rootstocks.

In the mid-2000s several new cultivars were imported by NZ Avocado including fruiting cultivars Carmen and Maluma, and rootstocks Dusa, Bounty and Latas. The imported cultivars are being evaluated at different levels in three replicated trials, in 11 semi-commercial plantings and in numerous commercial plantings.

Results from the replicated trials are confirming some of the traits observed overseas of the fruiting cultivars and confirming the superiority of some of the new rootstocks over the traditional rootstocks in replant situations. Results from the semi-commercial and commercial plantings are currently anecdotal. However, there are examples of commercial plantings on new clonal rootstocks that are being successful on virgin soil and in replant situations.

New government funding has allowed us to establish a new participatory research process to evaluate semi-commercial and commercial plantings in a systematic way. This process has widened the spread of our evaluation and will help us have a much better understanding of the value of the imported new cultivars for our industry.

■ Propagation of avocado seedlings of the cultivar “quintal” by grafts irradiated

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Due to difficulties of vegetative propagating in avocado, many tests were performed with ionizing radiation aiming the modification of the characteristics of the plants for obtaining of the improved genotypes and of smaller size. Rootstock “Nimlloh” cultivar of the Guatemalan race, were used to obtain avocado seedlings of “Quintal” cultivar. The cuttings were irradiated with different doses of gamma radiation: 0 (control), 10, 20 and 30 Gy, in a source of Cobalt-60 type Gammacell-220, installed in the Center for Nuclear Energy in Agriculture, CENA-USP. After irradiation, was performed the grafting type cleft graft. Were performed four evaluations of height of the plants from the budding grafting every 60 days. Was also calculated the average growth rate and volume of the plant canopy after 8 months. Data were subjected by statistic program (SAS) and the means were compared by Tukey test ($p < 0.05$). From the results obtained can be concluded that the dose of 10 Gy stimulated growth of the plants and 30 Gy reduced.

■ Utilization of two rootstocks for propagation of avocado seedlings of cultivars “Geadá” and “Fortuna”

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This first study aimed test a new method of propagation avocado seedlings “Fortuna” and “Geadá” cultivars from of two rootstock, as control was used one rootstock, seeking increase productivity and the resistance of the plant when there is soil humidity deficit. For the two tests were used 5 repetitions each. The experiment was conducted in greenhouse. After the grafting were made three evaluations of height (cm) from of the sprouting of graft every 60 days. Also was calculated the average growth rate and volume of the plants canopy after 8 months. For analysis of the data was utilized the statistic program (SAS) and the means were compared by Tukey test ($p < 0.05$). The results showed that the seedlings of avocado with one rootstock had a larger height and volume, when compared with the plants with two rootstock. This was expected because when use two rootstock the necrotic region takes longer time to occur the formation of new tissue. The same avocado seedlings utilized in first experiment were planted in the field to monitor the development of plants up to production.

■ Cotyledon mass reduction in the germination and initial growth of avocado

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The cotyledons are part of the avocado embryo and where reserves for germination are stored, however, it has proposed the elimination of a portion of the cotyledons, as a method to accelerate it, where there is doubt whether this affects the subsequent growth of plant. Germination and early growth of plants (113 days) due to cotyledon mass reduction (CM) without seed coats was studied. Avocado seeds of the Mexican race was used, but with some features of Guatemalan race, harvested from a single tree. 6 treatments with 4 replicates (24 seeds) were established. The treatments were completely randomized in a germination bed with a sandy substrate. The results showed that the number of buds, leaf dry weight, dry weight of cotyledons, weight of the aerial part, leaf area and total dry weight was higher for treatment with 100% CM. The dry weight of stems, dry weight of roots and stem diameter was higher for treatments with 100 and 75% of CM. Height to the first leaf was higher for treatments of 100, 75, 50 and 25% CM. The height to the first bud was superior in the treatment of 75% CM. The number of leaves did not differ between treatments. Plants that emerged first treatments were 75, 50 and 25% CM. Intact seeds did not germinate.

Diversity and distribution of the *Persea* genus in Mexico

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The possible distribution of *Persea* in Mexico is unknown, but species of this genus have been reported in 28 of the 32 states of the Mexican Republic, the scale of climate and physiographic variation of Mexico where they develop, can be found in other entities from the country. This investigation determined the possible distribution of the genus *Persea* in Mexico, as well as potential eco-climatic zones where different species of the genus are present, based on an analysis of geographic information systems (GIS), using a program called Maxent modeling. 19 climatic parameters and the known distribution of the species obtained from herbarium records and collections were evaluated. Most distribution of the genus *Persea* in Mexico is in areas of the Sierra Madre Oriental, Sierra Madre del Sur, Eje Neo Volcanico, Llanura Costera del Golfo, Sierras de Chiapas y Guatemala, y Peninsula de Yucatan. Groups of climatic distribution of the genus were three: the group I was formed by 16 species that grow in warm humid climates, semi moist, temperate humid and semi-cold; group II with 11 species in warm climate with humid, warm subtropical, temperate subhumid and semiarid; and group III with 10 species found in wet, humid and warm temperate semi-warm humid climate.

Effect of propagation and grafting technique on the morphological characteristics and root/shoot ratio in avocado plants

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Avocado plants [*Persea americana* Mill] of Duke 7 rootstock, propagated by seedling and through etiolation and layering technique, grafted and non-grafted with Hass variety, were planted in field and then extracted after a year in order to evaluate morphological characteristics of their root systems as well as biomass distribution in their aerial and root parts. This evaluation determined the effect of the propagation system used on morphological aspects of the root system such as more open angles of the main roots in clonal rootstocks as well as higher amount of absorbing roots –of first and second order- which increased root density by cubic meter of soil explored. In addition, grafting caused reduction in radical density and, in the case of seedling rootstocks, more open angles in the main roots, resulting in similar architecture between clonal and seedling rootstocks. However, grafted clonal rootstocks showed had higher root / shoot ratio.

Evaluation of new avocado rootstock selection for salinity conditions in irrigation water in Chile

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As a result of the avocado rootstock selection and propagation program, developed by the Propagation Laboratory of Escuela de Agronomía of PUCV, plant material resistant to irrigation water under salinity conditions was selected in the north part of our country. The most promissory, called UCV7 selection (West Indian race), was grafted with Hass variety and evaluated under salinity irrigation water, together with other rootstocks currently used in our industry such as Nabal seed (Guatemalan race) and clonal Duke 7 (Mexican race). One-year-old plants were established in containers under heated greenhouse conditions and were subject to irrigation with NaCl in different concentrations: 0.66mM (control treatment without additional NaCl); 6mM and 12mM. It was shown that the rootstocks have different strategies facing this stress. Duke 7 increased vegetative growth but, at the same time, the absorption of elements, causing a greater damage of ions Cl⁻ and Na⁺ in leaves (greatest damaged area). Both Nabal and UCV7 would not translocate Na⁺ to the area that would make them more resistant; however, UCV7 would show a considerable advantage compared to Nabal, since this exhibits a smaller damaged leaf area in high salinity conditions of irrigation water.

■ Clonal propagation of 'Duke' avocado (*Persea americana* Mill.) Using leafy cuttings with early roots in their etioled basal zone and individual humidity chambers

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Avocado cultivation in Yucatan is nascent, there ecotypes 'Creoles' showing some genetic variability and productivity. This germplasm has social and economic importance due to its market acceptance. Therefore, it is necessary to characterize these plant genetic resources and determine if they have any use in breeding success as avocado or commercial varieties. In this paper about the status and some outstanding local data Yucatan avocado germplasm we are presented. Yucatan avocado germplasm can be classified into three types, depending on your cycle: early, middle and late ripening. Highlights include Lagunero selections, Doble cosecha and Mulix (early); Suarez and Antillana (intermediate) and improved varieties Choquette, Christmas, Christmas Eve, Monroe, Collinson and Booth (late). Most avocado varieties present in Yucatán correspond to the West Indian, although there are some crosses with the Guatemalan race. Yucateco avocado germplasm even has potential to offer a staggered production throughout the crop year. However, lack of consistent evaluations mainly late varieties not allow avocado diversify production in Yucatan. The empirical selection of varieties for local producers to regional and national market, rather like "Lagunero" fruits. Therefore, consistent and systematic studies of such genetic resources of Yucatan to maximize their knowledge, conservation and use are required.

■ Characterization of regional avocado varieties (*Persea americana* var. *Americana* Mill.) in Yucatan, Mexico

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El cultivo de aguacate en Yucatán es incipiente, existen ecotipos 'criollos' que muestran cierta variabilidad genética y productividad. Dicho germoplasma tiene importancia social y económica debido a su aceptación en el mercado. Por ello, es necesario caracterizar dichos recursos fitogenéticos y determinar si tienen alguna utilidad en el mejoramiento genético del aguacate o éxito como variedades comerciales. En este trabajo se presenta información sobre el estado actual y algunos datos sobresalientes del germoplasma local de aguacate de Yucatán. El germoplasma de aguacate yucateco puede clasificarse en tres tipos, dependiendo de su ciclo: maduración temprana, intermedia y tardía. Destacan las selecciones Lagunero, Doble Cosecha y Mulix (tempranas); Suárez y Antillana (intermedias) y las variedades mejoradas Choquette, Navideño, Nochebuena, Monroe, Collinson y Booth (tardías). La mayoría de las variedades de aguacate presentes en Yucatán corresponden a la raza Antillana, aunque también se encuentran algunas cruza con la raza Guatemalteca. El germoplasma yucateco de aguacate incluso tiene potencial para ofrecer una producción escalonada durante todo el año de cultivo. Sin embargo, la falta de evaluaciones consistentes principalmente de las variedades tardías no permite diversificar la producción de aguacate en Yucatán. La selección empírica de variedades por productores locales para el mercado regional y nacional, prefiere frutos tipo "Lagunero". Por lo tanto, se requieren estudios consistentes y sistemáticos de dichos recursos genéticos de Yucatán para maximizar su conocimiento, conservación y aprovechamiento.

■ Molecular characterization and phylogenetic analysis of the *Persea* and *Eriodaphne* subgenera (Lauraceae)

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The genus *Persea* (Lauraceae) described in 1974 by Miller has about 90 species, delimitation within it has always been controversial. Molecular analysis have confirmed that the *Persea* group is monophyletic; however the recognition of two clades through phylogenetic analysis of morphological characters, suggests that the genus is not a monophyletic group and the *Eriodaphne* and *Persea* subgenera can be recognized as separate genera. Compatibility between some species of the *Persea* subgenus and the fact that most of its members grow from the central part of Mexico and Guatemala to Central America, supports the theory of the origin of the *Persea* genus and probably around the *Persea* subgenus too. The study of ITS regions indicated that the origin of the *Persea* subgenus is not monophyletic, the results grouped to the avocado species analyzed in different clades corresponding to the *Persea* and *Eriodaphne* subgenera, supporting the hypothesis of non-monophyletic origin of the *Persea* genus and providing an explanation to the incompatibility between the two subgenera. In order to help resolve the conflicts that arise within the group, we are analyzing 81 materials of *Persea* and *Eriodaphne* subgenera of the Universidad Autónoma Chapingo Avocado Germplasm Bank by SSR molecular markers and 21 intergenic regions of chloroplast genome, the results obtained will determine the phylogenetic relationships between materials and support if the origin of *Persea* is not monophyletic.

■ Identification of expressed sequence tags in fruit of mexican avocado race (*Persea americana* Mill. Var. *Drymifolia*)

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In the higher plants, the biological processes such as fruit ripening and senescence are regulated by a complex differential expression of genes. The identification, cloning and characterization are essential to understand these processes. The goal of this research were identify expressed sequence tags of immature fruits in mexican avocados (*Persea americana* Mill. var. *Drymifolia*) of Nuevo Leon in Mexico. Ten mexican avocado genotypes whose fruits have contrasting characteristics of shape and size, were selected for gene expression analysis. Procedures for detection of differentially expressed genes were optimized, 100 % of reproducibility in the amplified fragments were obtained. Ninety-four differential fragments were selected for sequencing, editing, identification and comparison with the NCBI nucleotide and protein databases. Of 82 sequences compared in the database only 40 sequences showed significant similarity with sequences of mRNA and/or hypothetical or predicted protein of *P. americana* and/or other genera. Some of the sequences were related to flavanone-3-hydroxylase (F3H) enzyme, pleiotropic drug resistance protein, lecithin-cholesterol acyl transferase enzyme and glutathione-S-transferase microsomal enzyme, others proteins did not show any defined function.

■ *Persea americana* Mill. embryos stored for six months remains viable for *in vitro* germination

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The *in vitro* culture of embryonic axis is effectively used in fruit species to obtain seedlings, genetic material rescue, and others. In vitro conditions allow the development and formation of healthy and axenic plants for micrografting, propagation, physiological studies and germplasm conservation. Particularly *P. Americana*, for being a monoembryonic specie, the rate of obtaining individuals by a single crossover are limited in addition to having reduced storage viability for its recalcitrance condition. Seeds from ripe fruit clone 'Duke 7' used as rootstock obtained from open-pollinated trees were disinfected in NaOCl (1.5%) and stored in the dark using plastic box closed by six months (10 ° C and 63% RH). Subsequently, in a laminar flow chamber, the embryonic axis were extracted and placed in culture medium of DFm (modified Dixon and Fuller 1976) and incubated in a growth room (22 ± ° C) and 89% relative humidity within the flask. The experiment was arranged in a completely randomized design with four repetitions of eight embryonic axes as experimental unit. The variance of the results was analyzed and compared the average (Tuckey p <0.05). After 65 days of incubation, 93% of structures remained viable, development of complete plants showed a quadratic response over the time (P = 0.022) as well as the explants in rosette status (P = 0.2303). Therefore, this research suggests that on aseptic storage, embryos of Duke7 clone remains viable for *in vitro* culture for six months.

■ Comparative seven varieties of avocado (*Persea americana* Mill.), on creole nursery level pattern in Tingo Maria

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Seven varieties of grafting avocado were evaluated. All of them were grafted into a nursery. The treatments were: T1: Villacampa, T2: Collinred, T3: La Molina-1, T4: Centro Oriental, T5: Choquette, T6: Hass and T7: Criollo. Completely Design Random was used in this experiment. We evaluated: Height, diameter of shoot and number of leaves. The result show that the Collinred (100%) and La Molina (86%) varieties had a good performance with the variety Criollo as a pattern.

Shoot induction of two cultivars of avocado *Persea americana* Mill.

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The microcuttings of two avocado cultivars were established on MS, DCR, Yasuda and B5 culture media, added with 2.0 mg L⁻¹ of BAP, 0.3 mg L⁻¹ of IBA, 2.0 g L⁻¹ of oxytetracycline and 2.0 g L⁻¹ of benomyl. The percentage of contamination, oxidation and viability of the explants were evaluated after two weeks in these conditions. Results showed that Yasuda for *in vitro* establishment generated the most surviving explants. The viable explants were subcultured in media shoot induction, which were MS, DCR and Yasuda supplemented with 20 % of coconut water (CW), 2.0 mg L⁻¹ of BAP, 0.5 mg L⁻¹ of GA3 and 0.01 mg L⁻¹ of IAA; 2.0 g L⁻¹ of casein hydrolysate (CH), 1.0 mg L⁻¹ of BAP and 0.3 mg L⁻¹ of IBA. The variables number and length of shoots, number of leaves at 8, 12 and 16 weeks were evaluated. T3 (Yasuda + CW) sowed the highest results with an average of shoot proliferation of 2.89 per explant at 16 weeks. Shoot growth ranged from 1.26 to 2.01 cm; and leaf development averaged of 3.18 leaves per shoot. The addition of casein hydrolysate in different culture media, did not favor the development of shoots in the cultivars used.

Difference of preservation and culture medium of embryos on *in vitro* germination of *Persea americana*

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The *in vitro* culture of embryonic axes has been used in most fruit species as support in plant breeding and obtaining of health plants. In explants of *Persea americana* different culture mediums have been referenced, including MS medium (Murashige and Skoog, 1962) and DF (Dixon and Fuller, 1976). In this research were evaluated the germination and development of embryonic axes of 'Duke 7' after extracted of seeds conserved for sixteen days at 10 ° C in plastic bags with and without holes in MS, MS30% and DF growth medium. Seeds were disinfested with sodium hypochlorite, rinsed and aerated at ambient conditions before being stored in the transparent bag; *in vitro* explants were kept in growth room (22 ± ° C, 89% relative humidity inside the flasks). The experiment was completely randomized and experimental unit consists of ten flasks each with a single explant. It was made ANOVA and Tukey comparison test. There was no interaction between the medium and storage type in survival percentage (P = 0.1452), with an average of 97% (cv= 5.72%) or rooting percentage (P = 0.2098) being, on average, 70% (cv = 27.09%). There was no interaction between factors on the percentage of explants rosette (P = 0.1074) but was different between medium, the lowest was observed in the MS (28%) as and was not different between MS30 and DF (52 %). None of the evaluated variables showed differences between the preservation in perforated or not bags.

Development of a protocol of infection root for quantification of genetic resistance to *Phytophthora cinnamomi* in avocado

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The root rot caused by the oomycete *Phytophthora cinnamomi* Rands is the main phytosanitary problem for the production of avocados in Colombia, causing losses between 30 and 50% of plants in nurseries and early crop establishment. The control of this disease has had limited success, where the genetic resistance of the rootstocks is seen as the most effective strategy. This research aimed to standardize a protocol following infection with *P. cinnamomi* in avocado patterns for use as a tool in quantifying genetic resistance in trade patterns. An experiment in nursery plants Hass avocados 2 months old from seed was established to determine the infective dose of the pathogen and the method of inoculation reproduces the root of disease symptoms. Hass avocado seeds were disinfested and germinated in sterile sand, after 20 days after emergence (DAE) transplanted into bags containing sterile substrate (peat, rice hulls, vermiculite and sand) in proportions of 3: 1: 1/2: 1/2 respectively. I mycelium grown wheat grains sterile isolation ARFRE-008 previously characterized as virulent as inoculum was used. Doses evaluated inoculum corresponded to 1, 2, 3, 5 g per kg of substrate, the half dose of the inoculum was applied to the substrate at transplanting to the bag and the other half when the plants reached 60 DDE. Treatment of injury to the root was also performed at the time of transplant public. Foliar symptoms were assessed at 8, 15 and 60 days after transplantation to bag and categorized according to the level of Gabor and Coffey (1991). Symptoms as percentage of visual root damage, line intercept technique, dry matter percentage *P. cinnamomi* recovery were evaluated at 60 after transplantation public. The trial was established in a design completely random (DBCA) blocks, factorial arrangement 2X4 (factor A = root with or without wound and factor B = dose of inoculum), with three replications and nine plants per experimental unit, each repeat had its corresponding absolute control. The data underwent analysis of variance and the averages compared by Tukey test (P <0.05). The results suggest that the dose of 5 g of inoculum is most suitable to reproduce the symptoms of disease and injury in the roots had no effect on the development of leaf symptoms, but if the symptoms and root development.

■ Determination of the degree of virulence in isolates of *Phytophthora cinnamomi* technique for injury to stem

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Intraspecific variability that occurs in the Oomycetes in the degree of virulence is necessary to characterize and select the most virulent strains for use in the selection of cultivars for genetic resistance, ensuring the selection of material with greater resistance. A collection of 25 isolates of *P. cinnamomi* from different growing regions in Colombia was purified avocado in half PARPH, characterized by macroscopic and microscopic morphological and PDA-preserved in sterile distilled water. An experiment graft nursery plants Hass avocado was established for categorizing isolates for virulence, using the technique of inoculation wound to stem segments of mycelium of the pathogen. The design was DCA, with six repetitions each isolate corresponded to a treatment and each repetition had its corresponding absolute witnesses. The length and width of the lesion were evaluated every three days from five days after inoculation (DDI) for 15 days, the lesion area (cm²) was obtained from these measurements. The data underwent analysis of variance and the averages compared by Tukey test ($P < 0.05$). The results indicated differences in virulence isolates.