REVISTA TEMAS AGRARIOS

ORIGINAL ARTICLE

Micrografting of Valencia orange and Tahiti lime

Microinjertación de naranja Valencia y lima Tahití.

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ABSTRACT

To determine the viability of micrografting of Valencia orange and Tahiti Lime plants on Cleopatra mandarin rootstocks for possible use in massive plant propagation, different steps o the micrografting procedure were evaluated. Seeds of Cleopatra mandarin were established in semisolid MS (Murashige and Skoog) with, or without, GA_3 to evaluate the effect on percentage and time time of germination. Plants of Valencia and Tahiti were, either, treated with 1 mg l^{-1} BAP or manually pruned to evaluate the effect on vegetative shoot production, and the effect of three positions of the shoot tip meristem (Topping, sided and slant) onto the decapitated surface of the rootstock on the percentage of success were evaluated. Treatments for all experiments were distributed with a complete randomized design and data for vegetative shoot production were analyzed with ANOVA and means separated with Tukey test. Results evidenced that GA_3 had no effect on increasing percentage and reducing time of germination, pruning statistically increased the number of vegetative shoots in plants. Micrografting success of 28% for Valencia orange and 14% for Tahiti lime are consistent with reported studies for recovery of disease-free plants; however, to increase success level for massive plant propagation, it is recommended for further studies to consider evaluation of *in vitro* conditions such as increased sucrose concentration, *in vitro* adaptation of shoot tip meristems and auxin supply, among others.

Key words: Citrus; *In vitro* germination; Shoot apical meristem; *In vitro* grafting.

RESUMEN

Para determinar la viabilidad de microinjertación en plantas de naranja Valencia y lima Tahití como un método de propagación masiva de plantas, diferentes pasos del proceso de microinjertación fueron evaluados. Las semillas de mandarina Cleopatra fueron establecidas *in vitro* en medio semisólido MS (Murashige y Skoog) con, o sin, GA_3, para evaluar su efecto en el porcentaje y el tiempo de germinación, las plantas de naranja Valencia y lima Tahití fueron tratadas con 1 mg l^{-1} BAP o podadas manualmente para evaluar el efecto sobre la producción de brotes vegetativos, y el efecto de tres posiciones del ápice meristemático en la superficie del patrón decapitado, en el centro, en un lado y en un corte inclinado, sobre el porcentaje de éxito fue evaluado. Las repeticiones de todos los experimentos fueron distribuidas con un diseño completamente al azar, los datos de brotes vegetativos fueron analizados con ANAVA y los promedios separados con la prueba de Tukey. Los resultados evidenciaron que el GA_3 no afectó el porcentaje y tiempo de germinación y la poda incrementó estadísticamente el número de brotes vegetativos. Los porcentajes de éxito de microinjertos de naranja Valencia (28%) y lima Tahití (14%) son consistentes con los resultados de otros estudios para recuperar plantas libres de enfermedades; sin embargo, para propagación masiva, se recomienda en futuros estudios considerar la evaluación de condiciones *in vitro* como aumento en la concentración de sacarosa, adaptación *in vitro* de los ápices meristemáticos y adición de auxina, entre otros.

Palabras clave: Citrus; Germinación *in vitro*; Meristemo apical; Injerto *in vitro*.

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INTRODUCTION

Citrus is one the most important group of plants in earth with a long history of cultivation originated from tropical and subtropical regions of southeast and spread toward Europe, Africa, America and Oceania (Wu *et al*., 2018). The genus Citrus belongs to the order of Geraniales, family Rutaceae, subfamily Aurantiodeae. The Rutaceae family includes about 160 genera and 1650 species consisting of trees and shrubs evergreen that grow and fruit at latitudes between 40º north and 40 º south; fruits are very rich in vitamin C, consumed fresh and transformed into juices, wedges, preserves, jellies and jams, and provide by products for cosmetics and medicinal products (Inglese and Sortino, 2019). Annual production of citrus in the world in 2018 was around 150 million ton where oranges accounted for 50%, mandarins 22%, lemons and limes 12% and other citrus fruits 6%. In Colombia, production in 2018 was estimated in 75000 ton distributed in 20% oranges, 13% mandarins, 14% limes and lemons and 52% other citrus fruits (FAO, 2020). In Córdoba department, citrus fruit production in 2018 was 1150 ton with more than 90% was orange production (Agronet, 2021). Citrus plants for planting commercial crops are usually propagated by grafting the desired cultivar onto selected rootstocks using "T" budding; rootstocks are usually selected for stress tolerance and growth habit, while grafts are usually selected for fruit quality and production (Wasielewsky and Balerdi, 2018; Albrecht *et al*., 2017; Baron *et al*., 2019). At the present there is a worldwide shortage in the supply of planting material due to new strict regulations to produce and commercialize citrus plants; these regulations include upgraded infrastructure to prevent pest arrivals and test to confirm negative diagnose for pathogens such as CTV (*Citrus tristeza* virus), CEVd (Citrus Exocortis Viroid) and HLB (Huanlongbing) (Vashisth *et al*., 2020; ICA, 2019) in both rootstock and grafts. Micrografting is an *in vitro* technique used to propagate plants by positioning an isolated shoot tip meristem on top of a decapitated rootstock; it combines the advantages of rapid *in vitro* multiplication with the ability to combine the genotypes desirable characteristics in a single plant (Suárez *et al*., 2005). Success of micrografting is associated with genotype, shoot tip source, rootstock culture and age, nutrient medium composition, sucrose concentration and graft technique. *In vitro* seed germination for micrografting can be affected for PGR (Plant Growth Regulator) supply in the medium; GA_3 (Gibberellic acid) is one of the most studied (Miceli *et al*., 2019; Iftikhar *et al*., 2019; Rout *et al*., 2017); while wedge and slit are the most common techniques used for micrografting fruit crops species (Rehman and Gill, 2015). In citrus, micrografting has been employed mostly as a technique to recover disease-free plants to be used as a source for massive propagation (Navarro *et al*., 1975; Kapari-Isaia *et al*., 2002; Singh, 2018). In the present research, the conditions for *in vitro* rootstock germination, plant management for shoot tip source and shoot tip meristem placement onto the decapitated rootstock were evaluated in the micrografting of two different, Valencia orange and Tahiti lime, citrus species.

MATERIALS AND METHODS

Plant material

Seeds for rootstock production were extracted from mature fruits obtained from field grown Cleopatra mandarin trees planted at the Universidad de Córdoba – Berastegui Campus (8º40´26" N 75º46´44" W). Fruits were washed twice with distilled water, hand-squeezed and seeds separated with a plastic sieve. Seeds were profusely washed with sterile-distilled water, air-dried overnight on filter paper and stored in sterile closed glass flasks during 4 weeks in a conventional fridge at 8 °C. Shoot tips were obtained from 2-year old grafted plants of Tahiti Lime and Valencia orange obtained from an

authorized citrus plant distributor (Reg. ICA 25290-06V) and maintained in a shade house (50%) with 5-min fog irrigation once a day.

In vitro rootstock germination

After storage, a total of 300 seed were scarified by seed coat removal, surface disinfected in a 1.05% active chlorine solution during 15 min and washed 3 times with sterile distilled water inside of a laminar flow hood. To evaluate the effect of GA_3 (gibberellic acid) on seed germination, half of the seeds (150) were established in test tubes (150 x 25 mm) dispensed with 30 ml of ½ MS (Murashige and Skoog, 1962) medium supplied with (in mg L^{-1}) sucrose (30000), myo-inositol (100), thiamine HCl (0.4), GA_3 (1) and TC-agar (6000). The remaining 150 seeds were established in medium of the same formulation with no GA_3 addition. Test tubes were capped with plastic caps, sealed with Parafilm® and placed in racks at 20 °C with 12-hour photoperiod (40 µmol m^{-2} s^{-2}) supplied with white cold fluorescent lamps. The experiment was a one-way factorial arrangement with 2 treatments (0 and 1.0 mg l^{-1} GA_3) and 150 replicates each. The experimental units (300) were distributed with a complete randomized design. The number of seeds germinated was registered daily and the percentage of germination calculated.

Shoot induction and growth in stock plants

To evaluate the effect of different treatments on shoot induction in 2-year old grafted plants to be used as shoot tip source, plants were either pruned or sprayed with 1 mg l^{-1} BAP (6-benzilminopurine). Terminal shoots, 4-5 cm from the apex, were removed in 6 plants of Valencia Orange and six plants of Tahiti Lime with a pruner. Simultaneously, the same number of different plants from both cultivars were sprayed with a solution at 1 mg l^{-1} BAP. Plants were maintained in a 50% shade house, with a 5-min fog irrigation a day. The experiment was of a two-way factorial (2 citrus cultivars and 2 GA_3 concentrations) with 4 treatments and 6 replicates per treatment for a total of 24 experimental units distributed with a complete randomized design. After 4 weeks, the number of new shoots was registered and analyzed with an ANOVA ($\alpha = 0.05$) and means were separated with the Tukey test ($\alpha = 0.05$).

Micrografting and plantlet recovery

The effect of three different shoot tip positions (Topping, in contact with central cylinder, chip budding, sided, and slant) on the success of micrografting of Valencia orange and Tahiti lime on *in vitro* germinated "Cleopatra" mandarin rootstock were evaluated. Shoot tips were obtained from 2-year old grafted plants maintained in a shade house with fog irrigation once a day. Stem sections (3-5 cm long) were surface disinfected in a 1.05% active chlorine solution and washed with three changes of sterile distilled water inside of a laminar Flow Hood. Rootstocks were individually germinated in germination medium consisting of semisolid MS (Murashige and Skoog, 1962) salt medium supplied with (in mg l^{-1}) sucrose (30000), myo-inositol (100), thiamine HCl (0.4) and TC-agar (6000). Rootstocks were decapitated at 2-3 cm height, and the shoot tips (<5 mm long) were placed as indicated for the shoo tip position. Micrografted plants were individually maintained in baby food jars dispensed with 30 ml of germination medium. Recipients were covered with 2 layers of heavy-duty aluminum foil and sealed with Parafilm®. Cultures were stored at 20 ºC with 12-hour photoperiod (40 µmol m^{-2} s^{-2}) supplied with white cold fluorescent lamps. The experiment consisted of a two-way factorial (2 citrus genotypes x 3 shoot tip positions) with 6 treatments and 50 replicates per treatment for a total of 300 experimental units distributed with a complete randomized design. After 10 weeks, the number of grafts with active shoot growth was registered and the success percentage

of the specific micrografting technique was calculated. Successfully micrografted plants were individually transplanted to *ex vitro* conditions in T53 tube recipients (12.5 cm x 3.4 cm) filled with peat as substrate. During the first week, plants were fog-irrigated 3 times a day, 5 min each. After 1 week, irrigation frequency was reduced to twice 5 min a day each, and finally after 2 weeks, plants were irrigated once a day with a 5 min irrigation. The number of plants that fully adapted to *ex vitro* conditions was registered for each treatment.

RESULTS AND DISCUSSIONS

In vitro rootstock germination

Cleopatra mandarin seeds germination began 10 after *in vitro* establishment and continue until day 20th (Figure 1A). A total of 125 (83.3%) seeds established in medium supplied with 1 mg l^{-1} GA3, germinated while 139 (92.7%) of the seeds established in medium without GA_3 germinated (Table 1). In both cases, maximum germination rate per day occurred at day 20th, and no further seed germination was observed beyond this day.

Table 1. Effect of GA_3 on *in vitro* germination of Cleopatra mandarin seeds.

Day after *in vitro* establishment	MS with 1 mg L^{-1} GA_3	MS without GA_3
10	10	15
11	7	2
12	18	1
13	4	5
14	3	3
15	4	15
16	16	24
17	0	6
18	7	7
19	27	9
20	29	52
Germinated seeds	**125**	**139**
Germination (%)	**83.3**	**92.7**

Citrus seed germination has been reported to be low in percentage and relatively time consuming (Prajapati *et al.*, 2017; Hussain *et al*., 2017). Pre-germination treatments such as GA_3 (Gibberellic acid) and scarification have been used in an attempt to increase germination rates and decrease the time for seed germination in several plant species (D´Este *et al*., 2019; Sayyad-Amin and Shahsavar *et al.*, 2019; Marler *et al*., 2019). GA_3 is a plant growth regulator well known to induce physiological response in plant processes such as germination, plant growth and development, and photosynthesis (Miceli *et al.*, 2019; Iftikhar *et al.*, 2019; Rout *et al*., 2017; Neelambari *et al.,* 2018; Shekafandeh *et al*., 2017). Results of citrus seeds treated with GA3 to improve seed germination have been inconsistent. Al-Janaby *et al*. (2016) achieved significant increase in germination up to 71.3%. of "Cleopatra" mandarin seeds treated with scarification and 500 mg L^{-1} GA_3. Khopkar *et al*. (2017) reported that "Pummelo" (*Citrus grandis* L. Osbeck) freshly collected seeds treated with 50 ppm GA_3 and heat at 50°C for 24 h resulted in significant increase in germination as well as more leaf area and root volume in seedlings compared to non-treated seeds. In contrast, Khan *et al*. (2002) evaluated the effect of 50, 300 and 500 ppm GA_3 on germination percentage and germination rate seeds of grapefruit (*Citrus paradise* Macf.), Kinnow mandarin (*Citrus reticulata* Blanco) and rough lemon (*Citrus lemon* L.) finding no effects of GA_3 on final germination percentage compared to control treatments. Al-Musawi and Al-Moussawi (2020) reported a gradual decrease in germination when acid lime seeds were treated with several concentrations (750 mg l^{-1} to 3000 mg l^{-1}) of GA_3 compared to water soaking as control treatment. Chaudhary *et al*. (2019) reported a reduction in time for seed germination and increased percentage of germination when seeds of Kagzi lime (*Citrus aurantifolia* Swingle)

were soaked in 500 ppm GA_3 for 12 hours. In the present research, seeds germinated in basic MS without GA_3 germination percentage was close to 10% higher compared to those germinated in medium added with GA_3.

Shoot induction and growth in stock plants

Shoot grew from axillar buds 1 week after pruning or BAP spraying. The ANOVA allowed to detect statistical differences (*Pr*< 0.0001) in the number of new shoots produced as a result of the treatments. Valencia Orange plants produced more shoots than Tahiti Lime plants regardless of the treatment (Table 2). For both cultivars, pruning resulted in higher number of induced and grown shoots than spraying with BAP. Tahiti Lime pruned plants induced 2x more new shoots than BAP sprayed plants, while Valencia Orange pruned plants produced 4x more shoots than Tahiti Lime plants treated with BAP (Table 2).

Table 2. Effect of pruning and BAP on average new shoot formation in stock plants of Valencia Orange and Tahiti lime

Treatment	Shoots
Valencia orange prunning	60.50 A*
Valencia orange BAP 1.0 mg l^{-1}	49.25 B
Tahiti lime prunning	31.25 C
Tahiti lime BAP 1.0 mg l^{-1}	14.50 D

*Number with the same letter are not different according to Tukey (α 0 0.05)

BAP (6-benzilaminopurina) is a synthetic PGR that promotes cell division and multiplication, promotes axillary shoot proliferation and reduce apical shoot growth; additionally, is commonly used in micropropagation (Akhtar *et al*., 2020). BAP between 1 and 5 mg l^{-1} is reported to significantly increase lateral shoot production in *Gmelina arborea* Roxb seedlings during stage 0 of micropropagation (Acosta, 2011). Amelia *et al*. (2020) reported that 5 mg l^{-1} BAP increased leaf number and shoot longitude in *Maleluca alternifolia* seedlings. On the other hand, pruning is a citrus crop activity intended to improve tree architecture, removal of unproductive and damaged parts, favor air flow and increase fruit quality (Chueca *et al*., 2021; Robinson, 2020; Vincent y Ritenour, 2020; Astiari *et al*., 2019). In the present research, new shoot promotion, by pruning or BAP spray to increase availability of plant material for grafting procedures, was evaluated observing that pruning produces more plant material (Shoots) and, eventually, reduce costs by eliminating PGR use.

Micrografting and plantlet recovery

Of the 300 plants that were *in vitro* grafted, 21 (14%) showed scion growth eight weeks after grafting was performed (Figure 1B). Graft success was observed only when shoot tip was placed in a slant position at the decapitated surface of the rootstock for both, Valencia orange and Tahiti lime shoot tips (Table 3). Shoot tip positioned at the top in contact with the central cylinder of the decapitated rootstock, or sided as a chip budding in contact with the cortex of the decapitated rootstock showed no success. It was observed that for some graftings, the decapitated rootstock area produced a callus tissue that overgrowth the grafted shoot tip (Figure 1C). Micrografted plants of Valencia Orange and Tahiti lime were transferred to *ex vitro* conditions; after 8 weeks of transplant, 7 (50%) plants of Valencia Orange and 3 (47%) of Tahiti lime survived and fully adapted to *ex vitro* conditions (Figure 1D).

Table 3. Effect of three methods of micrografting on Valencia orange and Tahiti lime

Shoot tip position	Cultivar	Number of micrografts	Successful micrografts	Success level (%)
Topping	Valencia	50	0	0
Sided	Valencia	50	0	0
Slint	Valencia	50	14	28
Topping	Tahiti	50	0	0
Sided	Tahiti	50	0	0
Slint	Tahiti	50	7	14
Total		**300**	**21**	**14**

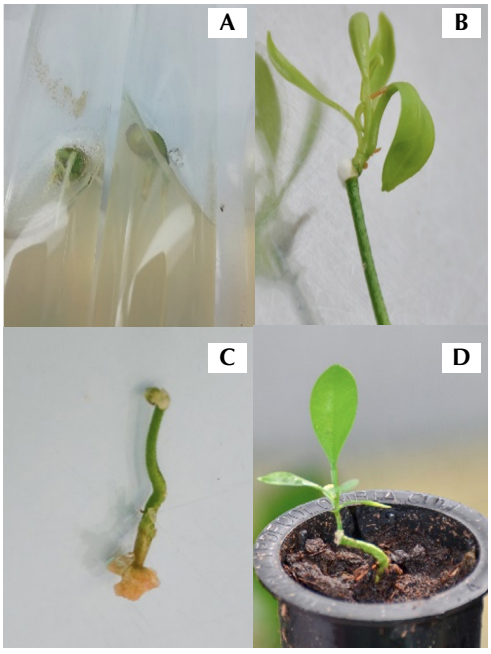

Figure 1. *In vitro* micrografting of citrus plants. **A** = *In vitro* germinated seeds, **B** = Micrografted plant of Valencia Orange, **C** = Overgrowth of callus tissue at the decapitated surface of rootstock, **D** = Micrografted plant adapted to *ex vitro* conditions.

Micrografting is a clonal propagation technique which involves the placement of a shoo tip meristem on top of a decapitated rootstock cultured *in vitro*; this technique is commonly used for recovering pathogen-free plants of citrus species (Hussain *et al*., 2014; Chand *et al*., 2013; Singh *et al*., 2018). In the present study, success level for micrografting ranged from 14% to 28%, slant positioned shoot tip was the only micrografting technique that allowed grafted plants recovery, and 50% of the successful grafts survived when transplanted to *ex vitro* conditions. Effect of shoot tip position on the decapitated rootstock seems to be genotype associated. Shridar and Venugopal (2019) reported highest success in pistachio when shoot tips were positioned in slant followed by wedge method; in contrast, for cherry plants cleft grafting resulted in better results. Micrografting technique seems to depend on several variables for successful graft union and plantlet recovery. Holding grafts and rootstock closely united until graft fusion is completed improve success level, and materials such as nylon band, aluminum foil tubes and filter paper have proven to be convenient (Obeidy and Smith, 1991). Naz *et al*. (2007) reported that increasing sucrose concentration in the semisolid MS (Murashige and Skoog) culture medium from 3% to 5% improved micrografting success from 21% to 33% in both, Kinoow mandarin and Succari oranges. Likewise, Parzaei *et al*. (2018) observed maximum micrografting success percentage when lime (*Citrus aurantifolia*) shoots were micrografted onto four rootstock genotypes cultured in MS medium supplied with 7.5% sucrose. Singh *et al*. (2018) reported that micrografting of Kashi mandarin resulted in maximum (56.8%) response when the rootstocks were cultured in semisolid MS medium fortified with 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} indole-3-acetic acid (IAA) along with 5% sucrose. Kanwar *et al*. (2019) found that culturing rootstocks of Carrizo citrange with 3 mg l^{-1} 2,4-D (2,4-diclorophenoxyacetic acid) resulted in maximum success (70%) and growth of grafted plants of sweet orange (*Citrus sinensis*) Cv. Red Blood. Success levels of micrografting obtained in the present research are low for massive plant propagation purposes; however, they are consistent with levels used as a technique for recovering disease–free plant material (Shridar and Venugopal, 2019; Kapari-Isaia *et al*., 2002). Strategies such as

modified medium components (Sucrose, PGRs, additives), size of shoot tips, type of recipient and height of rootstock decapitation will be addressed in future studies as a way to improve success levels.

CONCLUSIONS

The results of the present research allowed to conclude that germination of Cleopatra mandarin seeds *in vitro* was not improved by GA_3 addition in the medium, manual pruning induced a larger number of vegetative shoots in mother plants than BAP sprays and shot tip placement in slant position allowed recovery of micrografted plants for both, Valencia orange and Tahiti lime citrus species; however, more studies are recommended to improve micrografting success levels to be used as a mass plant propagation technique.

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Conflict of Interest

The authors declare that it is an original work and there was no conflict of interest of any kind in the elaboration and publication of the manuscript.

REFERENCES

Acosta C. 2011. Propagación *in vitro* de árboles superiores de melina (Gmelina arborea Roxb.). Tesis Magister. Universidad de Córdoba, Montería.pnas.1208652109.

Agronet, 2021. Resultados de las evaluaciones agropecuarias municipales del año 2017 del departamento de Córdoba https://www.agronet.gov.co/Paginas/ProduccionNacionalDpto.aspx

Amelia, Z., Supriyanto., Wulandari, A. 2020. Effect of 6-BAP application on shoot production of Melaleuca alternifolia seedlings. IOP Conference Series: Earth and Environmental Science, Orlando, Florida, October de 2021, 1-6p. https://doi.org/10.1088/1755-1315/528/1/012063

Al-Musawi, M., Al-Moussawi, S. 2020. GA 3 and Zn Impact on Germination and Seedling Growth of Acid Lime. Annals of Biology 36 (3): 406-411p.

Albrecht, U., Bordas, M., Lamb, B., Meyering, B. and Bowman, K. 2017. Influence of Propagation Method on Root Architecture and Other Traits of Young Citrus Rootstock Plants. Hortscience 52(11):1569-1576p. https://doi.org/10.21273/HORTSCI12320-17

Akhtar, S., Mekureyaw, N., Pandey, C., Roitsch, T. 2020. Role of Cytokinins for Interactions of Plants With Microbial Pathogens and Pest Insects. Journal Frontiers in Plant Science 10: 1-12p. https://doi.org/10.3389/fpls.2019.01777

Al-Janabi, A., AL-Sagheer, S. and Baekar, H. 2016. Effect of Mechanical Scarification, Gibberellic Acid and Chilling on Germination of Local Orange Seeds Citrus sinensis L. Journal of Biotechnology Research Center 10(1):38-44p.

Astiari, N., Sulistiawati, N., Mahardika, I., Rai, I. 2019. Improving the quality fruit of Citrus cv. Siam out off-season through the application of fertilization and pruning. IOP Conference Series: Earth and Environmental Science, Orlando, Florida, October de 2019, 1-6p. https://doi.org/10.1088/1742-6596/1402/5/055088

Baron, D., Esteves. A., Pina, A., Ferreira, G. 2019. An overview of grafting re-establishment in woody fruit species. Scientia Horticulturae 243:84-91p. https://doi.org/10.1016/j.scienta.2018.08.012

Chand, L., Sharma, S., Dalal, R., Poonia, A. 2013. *In vitro* shoot tip grafting in citrus species. A review. Agricultural Reviews 34(4):279-287p. https://doi.org/10.5958/j.0976-0741.34.4.013

Chaudhary, A., Ahlawat, T., Kumar, S., Jena, S., Patel, D. 2019. Effect of Gibberellic Acid on Germination and Vigour of Kagzi Lime Seedlings. Current Journal of Applied Science and Technology 38(6):1-8p. https://doi.org/10.9734/cjast/2019/v38i630411

Chueca, P., Mateu, G., Garcerá, C., Fonte, A., Ortiz, C., Torregrosa A. 2021. Yield and EconomicResults of Different Mechanical Pruning Strategies on "Navel Foyos"Oranges in the Mediterranean Area. Agriculture 11: 1-12p. https://doi.org/10.3390/agriculture11010082

D'Este, V., Axelsson, J., Yndgaard, F. and Solberg, S. 2019. Stratification, scarification and gibberellic acid treatments of garden angelica (Angelica archangelica) sedes Science and technology47(3): 243-247p. https://doi.org/10.15258/sst.2019.47.3.01

FAO (Food Agricultural Organization) 2020. Cultivos. Recovered from http://www.fao.org/faostat/es/#data/QC.

Hussain, G., Raja, N., Wani, Z., Mirl, M., Rather, Z., Bhat, K. 2014. Micrografting for fruit crop improvement. African Journal of Biotechnology 13(25): 2472-2483p. https://doi.org/10.5897/AJB2013.13602

Hussain, M., Raja, N., Maswani, Z., Iqbal, M., Ejaz, M., Yasmeen, F., Sohail. 2017. *In vitro* germination and biochemical profiling of Citrus reticulata in response to green synthesized zinc and cooper nanoparticles. IET Nanobiotechnology 11(7): 790 - 796p. https://doi.org/10.1049/iet-nbt.2016.0256

ICA (Instituto Colombiano Agropecuario). 2019. Resolución 0012816 (21/08/2019). https://www.ica.gov.co/getattachment/877b5bbb-0f20-4253-9f7d-7b20f03e2629/2019R01668.aspx.

Inglese, P., Sortino, G. 2019. Citrus History, Taxonomy, Breeding, and Fruit Quality. (Ed). Richard W. Hazlett, Oxford Research Encyclopedia of Environmental Science. https://doi.org/10.1093/acrefore/9780199389414.013.221

Iftikhar, A., Ali, S., Yasmeen, T., Saleem, M., Zubair, M., Rizwan, M., Abdulaziz, H., Alayafi, A., Soliman, M. 2019. Effect of gibberellic acid on growth, photosynthesis and antioxidant defense system of wheat under zinc oxide nanoparticle stress. Environmental Pollution. 254. https://doi.org/10.1016/j.envpol.2019.113109

Kanwar, I., Kaul, M., Naruka, I., Singh, P. 2019. *In vitro* micrografting technique in sweet orange (*Citrus sinensis*) cv. Blood Red to produce virus free plants. Indian Journal of Agricultural Sciences 89(3):494-499.

Khopkar, R., Nagaharshitha, D., Haldavanekar, P., Parulekar, Y. 2017. Studies on seed germination of pummelo (CITRUS GRANDIS L. OSBECK). International Journal of Agricultural 7(5):257-264.

Khan, M., Usman, M., Waseem, R., Ali, M. 2002. Role of gibberellic acid (GA3) on citrus seed germination and study of some morphological characteristics. Pakistan Journal of Agricultural Research 39(2): 113-118.

Kapari-Isaia, T. Minas, G. J. Polykarpou, D. Iosephidou, E. Arseni, S. Kyriakou, A. 2002. Shoot-tip Grafting *in vitro* for Eliminationof Viroids and Citrus psorosis virus in the Local Arakapas Mandarin in Cyprus. Fifteenth IOCV Conference, 2002, Short Communications International Organization of Citrus Virologists, p.417-419. https://escholarship.org/uc/item/0kk5h9ff

Marler, T. 2019. Serianthes nelsonii Seed Germination and Seedling Behavior are Minimally Influenced by Chemical and Light Treatment. Horticulturae 5(2), 1-7. https://doi.org/10.3390/horticulturae5020031

Murashige, T., Skoog. F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Miceli, A., Moncada, A., Sabatino. L., Vetrano, F. 2019. Effect of Gibberellic Acid on Growth, Yield, and Quality of Leaf Lettuce and Rocket Grown in a Floating System. Agronomy 9(7): 1-22p. https://doi.org/10.3390/agronomy9070382

Navarro, L.; Roistacher, C. N., Murashige, T. 1975. Improvement of shoot-tip grafting *in vitro* for virus-free citrus. Journal of the American Society for Horticultural Science 100(5):471-479.

Naz, A., Jaskani, M., Abbas, H., Qasim, M. 2007. *In vitro* studies on micrografting technique in two cultivars of citrus to produce virus free plants. Pakistan Journal of Botany 39(5): 1773-1778.

Neelambari., Mandavia, C., Sree, S. 2018. Curative Effect of Ascorbic Acid and Gibberellic Acid on Wheat (Triticum astivum L.) Metabolism under Salinity Stress. International Journal of Current Microbiology and Applied Sciences 7(1): 522-533p. https://doi.org/10.20546/ijcmas.2018.701.063

Obeidy, A., Smith, M. 1991. A Versatile New Tactic for Fruit Tree Micrografting. Americam Society for Horticultural Science 4: 91-95p. https://doi.org/10.21273/HORTTECH.1.1.91

Prajapati, DG., Satodiya, BN., Desai, AB., Nagar, P. 2017. Influence of storage period and growing media on seed germination and growth of acid lime seedlings (*Citrus aurantifolia* Swingle) Cv. Kagzi. Journal of Pharmacognosy and Phytochemistry 6(4): 1641-1645.

Robinson A. 2020. Pruning Principles for High-Quality Citrus. https://citrusindustry.net/2020/11/23/pruning-principles-for-high-quality-citrus/

Rout, S., Beura, S., Khare, N., Patra, S., Nayak, S. 2017. Effect of seed pre-treatment with different concentrations of gibberellic acid (GA3) on seed germination and seedling growth of *Cassia fistula* L. Journal of Medicinal Plants Studies 5(6). 135-138.

Rehman, HU., Gill MIS. 2015. Micrografting of Fruit Crops-A Review Journal of Horticulture 02(03): 1-7p. https://doi.org/10.4172/2376-0354.1000151

Singh, K. 2018. Propagation of citrus species through cuttings: A review. Journal of Medicinal Plants Studies 6(1):167-172.

Suárez, IE., Schnell, RA., Kuhn, DN., Litz, R. 2005. Micrografting of ASBVd-infected Avocado (Persea americana) plants. Plant Cell Tissue and Organ Culture 80: 179-185p. https://doi.org/10.1007/s11240-004-1622-3

Sayyad-Amin, P., Shahsavar, A. 2019. Improvement of Seed Germination of Date-plum (*Diospyros lotus* L.) by Physical and Chemical Treatments. Journal of Chemical Health Risk. 9(1): 51-56. https://doi.org/10.22034/JCHR.2019.664164

Shekafandeh, A., Sirooeenejad, S., Alsmoushtaghi E. 2017. Influence of Gibberellin on Increasing of Sodium Chloride Tolerance via Some Morpho-Physiological Changes in Two Olive Cultivars. Agriculturae Conspectus Scientificus 82(4): 367-373.

Vashisth, T., Chun, C., Ozores, M. 2020. Florida Citrus Nursery Trends and Strategies to Enhance Production of Field-Transplant Ready Citrus Plants. Horticulturae 6(1):1-15. https://doi.org/10.3390/horticulturae6010008

Vincent, C., Ritenour, M. 2019. Optimize Fresh Fruit Results With Selective Pruning. Citrus Industry. https://citrusindustry.net/2019/08/07/optimize-fresh-fruit-results-with-selective-pruning/

Wasielewski, J. and Balerdi, C. 2018. Types of asexual propagation. Tropical and Subtropical Fruit Propagation, Institution. UF/IFAS Extension, University of Florida, Institute of Food and Agricultural Sciences. https://edis.ifas.ufl.edu/pdffiles/HS/HS134900.pdf.

Wu, G., Terol, J., Ibanez, V., López-García, A., Pérez-Román, E., Borredá, C., Domingo, C., Tadeo, F., Carbonell-Caballero, J., Alonso, R., Curk, F., Du, D., Ollitrault, P., Roose, M., Dopazo, J. Gmitter, F., Rokhsar, D., Talon, M. 2018. Genomics of the origin and evolution of Citrus. Nature 554:311-316. https://doi.org/10.1038/nature25447