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Essay

Germination of tropical seeds *Pouteria campechiana*

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Abstract

Pouteria campechiana (Kunth) Baehni, commonly known as canistel or yellow sapote, is a perennial fruit tree in the Sapotaceae family. This plant is used in Mexico and Central America as an ethnomedicinal; it has an important role in different biological activities; the main biochemical components of this species are flavonoids, polyphenols, and carotenoids. The review aimed to present and analyze the information generated on the germination and development of the *P. campechiana* plant until the transplantation to the final field. This was with the intention of summarizing the knowledge of how to reproduce *P. campechiana* plants that allows promoting the use of this biotic resource. This review elucidates the strategies for *ex vitro* propagation, the manual scarification technique, and the seeding position of the seed that promote the development of the root and plumule and how malformed root pruning improves the development of the plant, as well as the optimization of propagation and how root pruning of *P. campechiana* plants before transplanting benefits the natural association with arbuscular mycorrhizal fungi; all this with the aim of improving the production of quality plants and the nutritional and therapeutic application of *P. campechiana*. Critical information about the discoveries and propagation strategies and their effect on the production of this multipurpose tree, which would help in the study of plants that have been underutilized but have potential.

Keywords:

canistel, development, growth, yellow sapote.

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Pouteria campechiana (Kunth) Baehni is a species of the Sapotaceae family, native to Mexico, with food, medicinal, ornamental, and timber properties; for its pleasant flavor, the fruit is considered exotic and is highly appreciated for consumption. In Florida, USA, they produce the fruits of *P. campechiana* and are marketed at local fairs for fresh consumption and processed into ice cream, shakes and bakery (Lim, 2013).

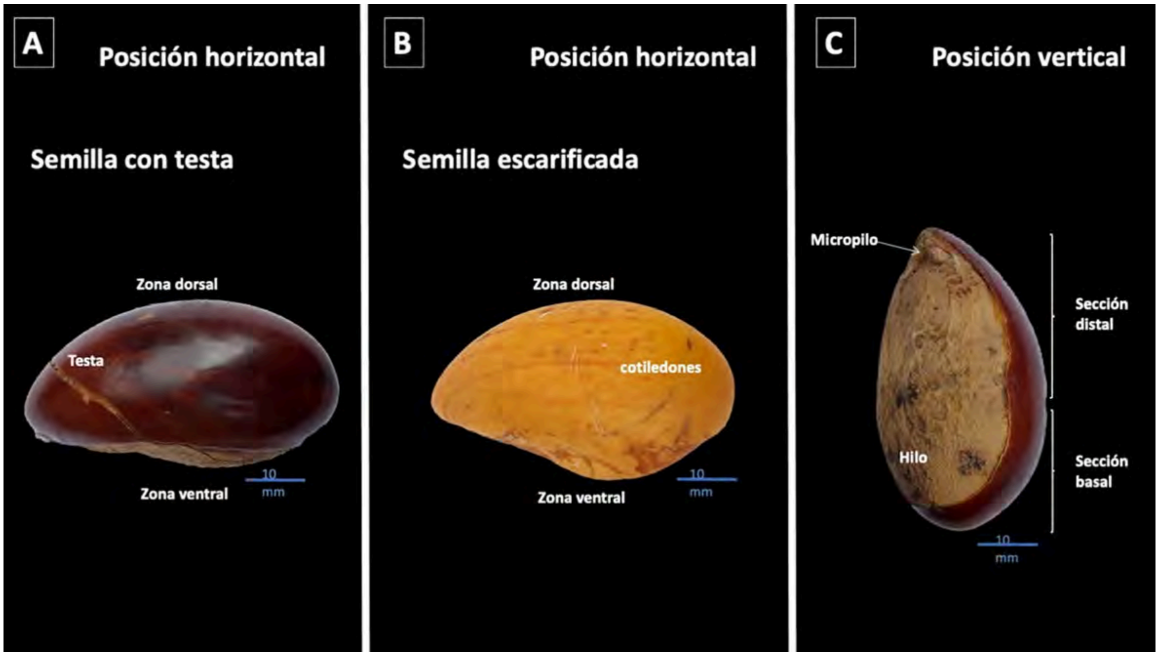
In Mexico, there are no commercial plantations and *P. campechiana* trees are located in backyard crops and the fruits are marketed in the local market (Pérez-Barcena *et al*., 2021a); the trees that are distributed in the country come from seed; nevertheless, it is also possible to propagate them through cuttings (Chiamolera *et al*., 2014).

The *P. campechiana* tree is evergreen, reaching 25 m in height with a straight stem and sympodial branching (the lateral branches develop more than the main axis), the bark of the trunk is brown, rough, and with abundant white and rubbery latex (Azurdia, 2005). The leaves are elliptical with an acute or rounded apex and the midrib protrudes and has 9 to 18 pairs of secondary veins.

The fruits have a pyriform shape and their length ranges between 6.5 and 7.5 cm; in the immature state, the peel is green and the pulp is hard and rubbery, when ripe, the peel turns yellow-orange and the pulp has a doughy and soft consistency and the fruit contains one to five seeds (Pennington, 1990).

The seeds of *P. campechiana* are protected by a thick bright dark brown testa (Figure 1A), which distinguishes the dorsal part of the seed and the large rough thread on the ventral part with a light hue (Figure 1C) (Martín and Malo, 1978; Awang-Kanak and Bakar, 2018). When the testa is removed, the pinkish-yellowish seed can be seen (Pérez-Barcena *et al*., 2021a) (Figure 1B).

Figure 1. ***Pouteria campechiana*** **seed, it shows the areas and positions (Pérez Barcena** ***et al*.****, 2021a). a) Horizontal position of the seed with testa; b) horizontal position of the seed without testa and c) vertical position of the seed with testa.**



Selection of *Pouteria campechiana* seeds for propagation

In the selection of *P. campechiana* seeds for propagation purposes, it is not necessary to separate the larger fruits since the physical characteristics of the seeds are similar; in a study in which they separated fruits of different sizes, Pérez-Barcena et al. (2021a) concluded that the size of the seeds and the percentage of germination were similar.

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Germination of *P. campechiana* seeds

Seed germination is the process of reactivating the metabolism of the embryo and the appearance of the structures that define germination, emergence, and development; it begins with the appearance of the radicle, lateral roots, and plumulae (shoot with vestigial leaves), followed by the development of the plant (Nonogaki, 2008).

The seed of *P. campechiana* is recalcitrant and a high percentage of germination (95-98%) is obtained from the seeds without prior storage, from the removal of the testa (manual scarification) and from sowing the seed with the ventral region buried 5 cm in the substrate and the dorsal region superficial to the substrate; germination is semi-hypogeal; in some tropical species with large seeds, such as *P. campechiana*, the seedling remains attached to the seed and the cotyledons or embryonic leaves remain attached to each other.

In addition, when the seed is attached to the seedling, it has photosynthetic potential (it turns greenish when the plumule appears with vestigial leaves); the seed is mummified when the reserves are exhausted (more than 120 days after germination) (Pérez-Barcena *et al*., 2021a). In this germination process, there is a replacement in the macromolecules of seed reserve as a response to the environment where germination, emergence, and development of the plant occur.

This review aimed to present and analyze the morphological information, data related to biochemistry, and techniques generated on the germination and development of the *P. campechiana* plant until the transplantation to the final field. This was with the intention of reproducing *P. campechiana* plants and favoring the use of this biotic resource. Bautista *et al*. (2022) comment that research on seeds is necessary in Mexico, which will allow greater knowledge of the areas of seed sciences for the diet and maintenance of the germplasm of Mexican species.

Macromolecules involved in the germination of *P. campechiana* seeds

Macromolecules are biological substances that have various functions in plants and seeds. In seed germination, the main macromolecules involved are reserve carbohydrates and proteins, which accumulate in the cotyledons and constitute the source of matter and energy from germination to seedling development, when its photosynthetic process is already activated (Rajjou *et al*., 2012).

Carbohydrates, such as starch and sugars that are stored in the seed, are a source of energy for the growing embryo; these carbohydrates are broken down into simple sugars by the action of enzymes (Bewley *et al*., 2013). Nucleic acids: such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), they are involved in regulating the expression of different genes that lead to the production of proteins and other molecules that are precursors for the growth of plant tissues.

Proteins: they are important for the growth and development of the seedling; enzymes called proteases are activated in germination and they transform the stored proteins into the different amino acids that compose them, amino acids can be used for the synthesis of new proteins and enzymes required for plant growth (Kumar *et al*., 2021).

Lipids: such as oils and fats, they are also macromolecules that are stored in the seed and serve as a source of energy in the course of the germination process (Nonogaki, 2008). In general, the catabolic and anabolic reactions of the macromolecules stored in the seed are decisive for seedling growth and successful tree development.

Carbohydrates

The carbohydrates in the seed are mainly found in the form of starch; this reserve polysaccharide abounds in the endosperm of the seed and as the germination process progresses, it is catabolized

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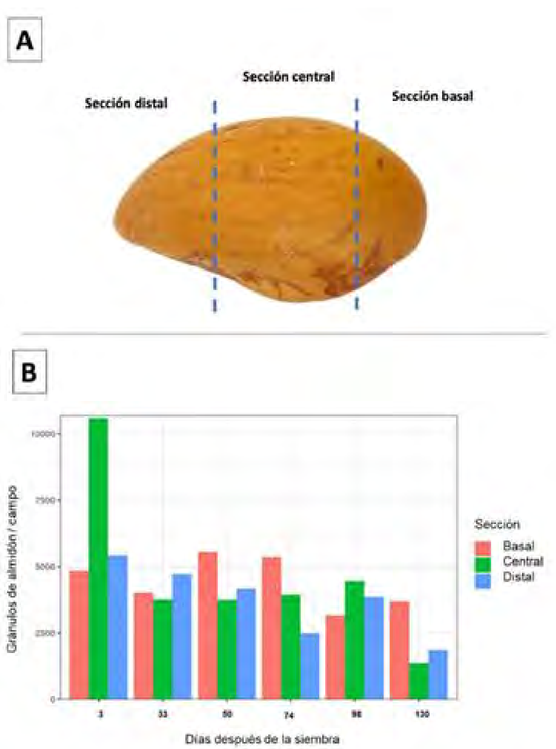
to generate the necessary energy in the anabolic reactions that are needed in the synthesis of new macromolecules essential for the growth of the young tissues of the plant. Starch is a polysaccharide composed of two types of polymers: amylose and amylopectin; this compound is present in all plants and is the main reserve energy in them (Agama-Acevedo *et al*., 2013).

During the germination process, when it comes into contact with water, metabolism is activated. The seeds of *P. campechiana* are rich in starch, so much so that it is proposed to use it in the diet; the starch isolated from the seeds of immature fruits has long chains of amylopectin that give it a thermally stable helical structure (high percentage of crystallinity and degree of polymerization), which gives it the characteristic of resistant starch; after cooking, it shows an increase in the content of slow-digesting starch (Agama-Acevedo *et al*., 2023).

Research, such as Granados-Vega *et al*. (2022), reported, through histological, microscopic, and imaging techniques, how the use of starch granules occurs during the germination process of *P. campechiana* seeds (Figure 2); starch granules are stored in greater quantities in the central zone of the seed and in a smaller proportion in the basal and distal zones (Figure 2A). During the germination process, starch granules from the central and basal zones are mostly consumed (Figure 2A-B).

Figure 2. A) seed of *Pouteria campechiana* without testa, signaling of the basal, central, and distal regions and B) distribution of starch granules from *P. campechiana* seed in the different sections of the seed during its germination process.



Lipids

Lipids are found in a lower or higher proportion depending on the type of seed; in *P. campechiana*, the lipid content in the seed is 1.7% (Evangelista-Lozano *et al*., 2021). The above differs from *Pouteria sapota* (mamey), which contains 44.41% lipids, among which are oleic, stearic, palmitic, and linoleic acids (Solís-Fuentes *et al*., 2015).

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Lipid hydrolysis is in charge of lipase enzymes; these hydrolytic enzymes are associated with lipid catalysis during seed germination and in turn are directly related to various hydrolytic processes; the reserve lipids are hydrolyzed as the germination process progresses. Regularly, the reserve lipids are contained as triacylglycerols (TAGs), which are catalyzed by these enzymes to release free fatty acids, glycerol, tocopherols, triglycerides, and phospholipids (Kumar *et al*., 2021).

Mobilization of reserves

The seeds of *P. campechiana* store reserve substances, mainly in the endosperm (Evangelista-Lozano *et al*., 2021). During the germination process of *P. campechiana*, starch is the main energy reserve and is contained in the central and distal parts of the seed, as the germination process progresses, it degrades by mobilizing the reserves towards the basal part of the seed where the embryo is waiting for energy to start the elongation process, the emergence of the radicle and plumule (Figure 3); a seed of *P. campechiana* becomes a plant in an average of 52 days after establishment (Pérez-Barcena *et al*., 2021a).

Figure 3. *P. campechiana* plant under development.

Water imbibition

Imbibition is the first phase where germination is triggered, which leads to the growth of the embryonic axis and the appearance of the radicle (Azcón-Bieto and Talón, 2008), with the activation of protein synthesis and resumption of respiratory activity (Bewley *et al*., 2013).

The sexual reproduction of plants involves metabolic and molecular mechanisms that can be summarized as: 1) imbibition of water by the seed; 2) activation of metabolism, respiration process, protein synthesis, and mobilization of reserve substances and 3) elongation of the embryo and rupture of the testa through which the radicle emerges (Suárez and Melgarejo, 2010).

The reserve tissues (endosperm) of recalcitrant tropical seeds imbibe water until hydration is complete (Moreno *et al*., 2006). In the case of *P. campechiana*, it is reported that scarified seeds show a higher germination speed (2.05 seeds per day); the above is because, as they do not present testa, they imbibe water more efficiently and initiate the germination process; seeds with testa showed a germination speed of 0.22 seeds per day (Pérez-Barcena *et al*., 2021a).

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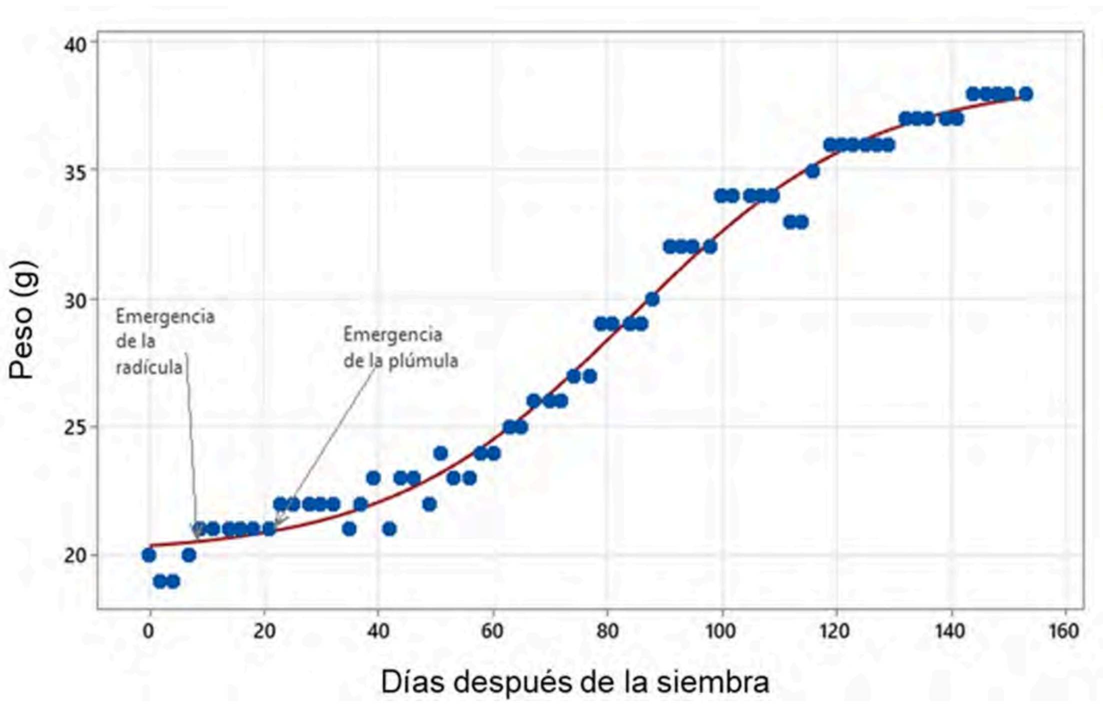
These results coincide with those reported by Granados-Vega *et al*. (2021), who observed, in *P. campechiana* seeds, that the germination capacity of scarified seeds was 95% in 10 days after sowing; in addition, 90 days after sowing, they generated plants of 19.4 cm in height, a stem of 0.4 cm in diameter, and an average of seven leaves, ready to be transplanted in the field or in a larger container; they also present a curve in which the three phases are observed: phase 1 imbibition; phase 2 lag (metabolic activity), and phase 3, in which the elongation of the root and aerial part occurs (Figure 4).

Figure 4. Growth and development curve of *P. campechiana*. From germination to fully developed plants (Granados-Vega *et al*. 2021).



The germination of *P. campechiana* seeds is influenced by the sowing position because the embryo is located in the basal region of the seed (wide part of the seed); there is a high percentage of germination in (scarified) seeds sown in a horizontal position three days after harvest compared to those sown vertically (Pérez-Barcena *et al*., 2021b).

Induction of germination in *P. campechiana*

In this sense, Amoakoh *et al*. (2017) report that seed dormancy and low germination of *P. campechiana* are the main problems in propagation; they obtained the highest percentage of germination in moderately scarified seeds (67%) compared to fully scarified seeds (63%) and non-scarified seeds 57%; this research does not mention what type of fruit they used to extract the seeds, nor the days after the harvest when they sowed; Andrade *et al*. (2002) studied the effect of temperature on the percentage of germination in *P. campechiana* seeds, and the highest germination (89%) was obtained at 30 °C.

The sowing position in germination is important because of the place where the embryo is; this facilitates the emergence and development of the root and plumule, as reported by Duarte and Suchini (2001) for *Pouteria sapota*. In addition, Pérez-Barcena *et al*. (2021a) also reported high germination percentages in *P. campechiana* seeds. These same authors point out the importance of considering the days after harvest to obtain quality seeds, fruit ripeness, total scarification, and planting position.

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Root pruning in *P. campechiana*

The root system is considered a plant organ and has communication with the aerial parts of the plant through the stem; it is a transport system of substances, such as photoassimilates, phytohormones, water, ions and signaling molecules, which allow the maintenance and development of the plant; therefore, the integrity of the root system has effects on the vigor of the plants and must be kept in optimal conditions (Bengough *et al*., 2011).

Root development is determined by biotic factors, interactions between the root and other organisms, either antagonistic, such as herbivores, or mutualists, as in the case of arbuscular mycorrhizal fungi, and abiotic factors, which include soil composition, such as water availability, porosity, degree of compaction, its specific composition of clay, silt, and sand, pH and the presence or absence of minerals (Tuzzin *et al*., 2018).

Root growth is also exposed to frictional forces and impedance pressures as it passes through soil horizons, pores, other organisms, and physical barriers (Yan *et al*., 2017). Physical barriers change the morphology of the root due to mechanical impedance. It is defined as the physical resistance of the soil to root expansion or dispersal (Kerk and Sussex, 2012).

The effect of mechanical impedance causes deformations in the roots, which can include winding (root segments that wrap around a stem or other root segments), curling (bending of the root, resulting in the formation of strong angles), and clustering (due to compaction of root segments) (Yan *et al*., 2017), which leads to a worsening of plant deformations due to greater compaction that decreases the absorption capacity, architecture, and root system; therefore, plants die early when transplanted in the field (Gilman *et al*., 2010).

To control malformations, root pruning is used, which is an agricultural practice that improves plant development and performance by promoting lateral root development (Arnold, 2015). In *P. campechiana*, Galvis-Muñoz *et al*. (2019) show the effect of mechanical pruning of malformed root segments on root growth, development, and architecture in plants ready to go to the field (6 months after sowing); at 80 days after transplanting in a sandy loam soil with pH of 5.7, the results were a greater number of leaves, organized root structure, length of the ramifications and coverage of root hairs compared to the control; therefore, pruning malformed roots before transplanting to the field is an excellent practice in *P. campechiana*.

Arbuscular mycorrhizal fungi in *P. campechiana* plants

The roots of higher plants are associated with arbuscular mycorrhizal fungi (Rodrigues and Rodrigues, 2014); this is practically obligatory due to the need for the fungus to complete its life cycle, and 80% of terrestrial plants are recorded with one or more species of arbuscular mycorrhizal fungi (Souza, 2015). Pham (2008) findings report that plants of the Sapotaceae family do not form mycorrhizae; authors such as Carmona *et al*. (2013) report that there is an association of wild plants of *Bumelia retusa* L. (family Sapotaceae) with arbuscular mycorrhizal fungi; Padilla *et al*. (2006) also report it for *P. lucuma* (synonym of *P. campechiana*).

Galvis-Muñoz *et al*. (2019) conducted a study on six-month-old *P. campechiana* plants with malformed root pruning, which were evaluated at 80 days after transplantation in the field, and they reported that small trees with root pruning presented greater natural association with arbuscular mycorrhizal fungi, showing a statistically significant difference from 50 days after transplantation in terms of the percentage of frequency and intensity of colonization (Figure 5).

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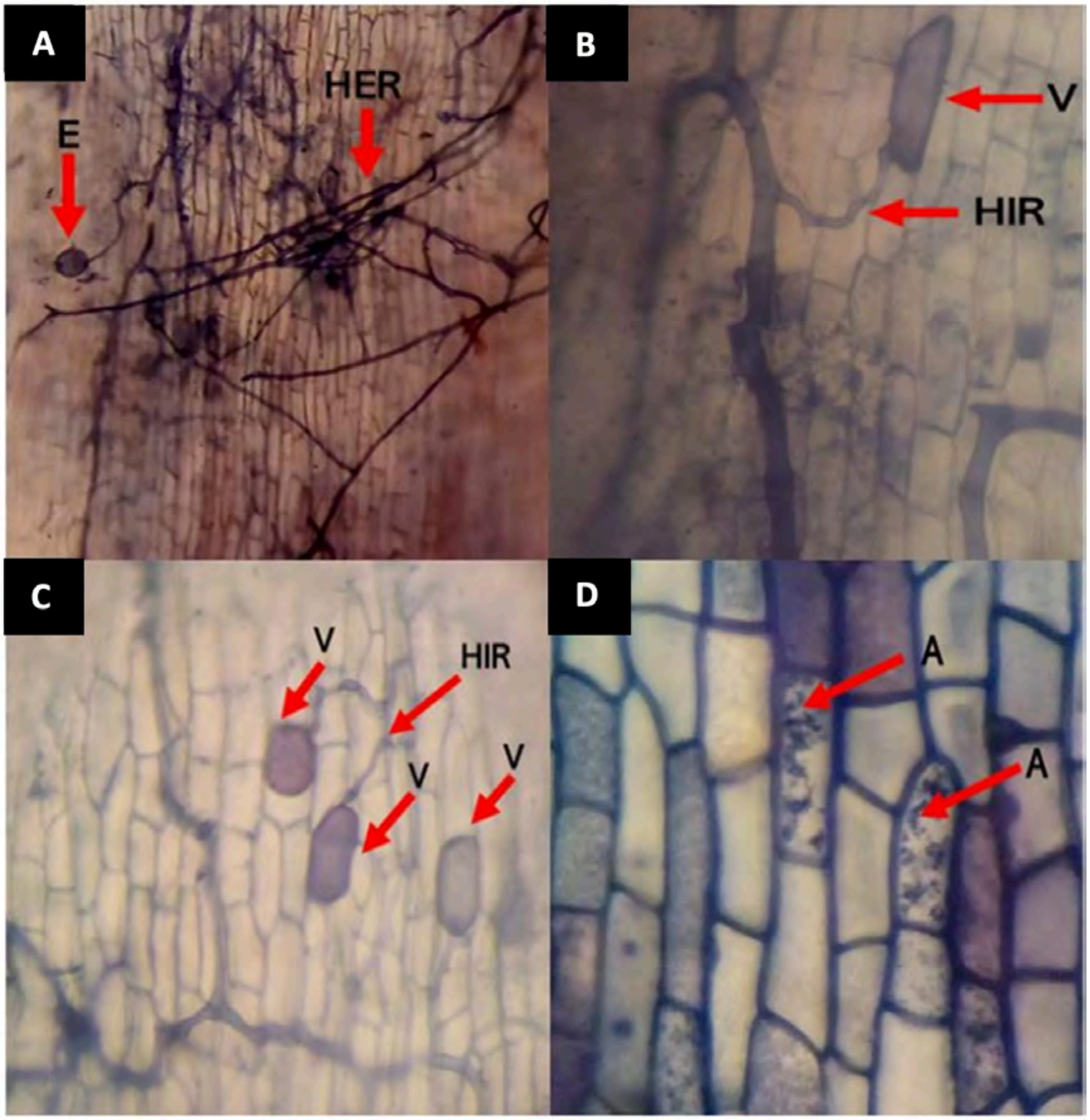
Figure 5. Mycorrhizal structures in P. campechiana roots. A= E spore, HER germ tube connected to the network of extra-radical hyphae; B= V vesicle, HIR network of intraradical hyphae; C= V group of interconnected vesicles, HIR network of intraradical hyphae; D= A arbuscules inside the cells of the cortex. (Saplings with root pruning before transplanting to the field and collected 80 days after planting) (Galvis-Muñoz *et al*., 2019).



Conclusions

This review of the germination and development process of *P. campechiana* highlights the variation in the presence of macromolecules in the seeds of Sapotaceae during the germination process. During seed imbibition, the phases that give rise to a growth and development curve take place. The sowing position of the seed promotes the development of the root and plumule of the *P. campechiana* plant. Malformed root pruning enhances the development of *P. campechiana* plants. Root pruning of *P. campechiana* plants before transplanting favors natural association with arbuscular mycorrhizal fungi.

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