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Bibliographic review

Micropropagation of *Vachellia* spp. as an alternative for the reforestation of desert areas in Mexico

Micropropagación de *Vachellia* spp. como una alternativa para la reforestación de zonas desérticas en México

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ABSTRACT: The irrational exploitation of native species of arid and semi-arid areas of Mexico, in particular the huizache (*Vachellia* spp.), created the need to carry out research work that contributes to the reforestation of these areas. *Vachellia* spp. pods have been characterized by their nutritional value and use in ruminants, in addition to the fact that generally the seeds present germination problems under natural conditions, due to their hard and impermeable cover that prevents the passage of water. This is a problem that can be solved through the use of micropropagation. This technology has made it possible to obtain thousands of disease-free seedlings with characteristics identical to the plant that gave them origin, and so far there is no extensive research on the subject that includes diversity of species of the genus *Vachellia* L., some of the few examples are the case of the efficient induction of callus of the species *Vachellia macracantha*, induction of shoots and multiplication of *Acacia auriculiformis*, *in vitro* propagation of *Acacia chundra* and the hybrid *Acacia mangium* and *A. mangium* × *A. auriculiformis* hybrids, among other methodologies. Scarification methods (physical, chemical and mechanical) interrupt the dormancy of the seeds and by placing them in a suitable culture medium (macro and micronutrients, sucrose, growth regulators), large productions have been obtained in a short time. The aim of this Bibliographic Review was to compile the most relevant results on plant tissue culture in species of the genus *Vachellia* L.

Key words: biotechnology, plant tissue culture, arid and semi-arid zones.

RESUMEN: La explotación irracional de especies nativas de zonas áridas y semiáridas de México, en particular el huizache (*Vachellia* spp.), creó la necesidad de realizar trabajos de investigación que contribuyan a la reforestación de dichas zonas. Las vainas de *Vachellia* spp. se han caracterizado por su valor nutricional y uso en rumiantes; además, generalmente las semillas presentan problemas de germinación en condiciones naturales, debido a su cubierta dura e impermeable que impide el paso del aguA. Esto es una problemática que se puede solventar a través del uso de la micropropagación. Esta tecnología ha permitido la obtención de miles de plántulas libres de enfermedades y con características idénticas a la planta que les dio origen y, hasta el momento, no existe una amplia investigación en la temática que incluya diversidad de especies del género *Vachellia* L.; alguno de los pocos ejemplos es el caso de la

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inducción eficiente de callos de la especie *Vachellia macracantha*, inducción de brotes y multiplicación de *Acacia auriculiformis*, propagación *in vitro* de *Acacia chundra* y del hibrido *Acacia mangium* y *A. mangium* × *A. auriculiformis hybrids*, entre otras metodologías. Los métodos de escarificación (física, química y mecánica) interrumpen la dormancia de las semillas y al colocarlas en un medio de cultivo adecuado (macro y micronutrientes, sacarosa, reguladores del crecimiento) se han obtenido grandes producciones en corto tiempo. El objetivo de esta Reseña bibliográfica fue recopilar los resultados más relevantes sobre el cultivo de tejidos vegetales en especies del género *Vachellia* L.

Palabras clave: biotecnología, cultivo de tejidos, especies nativas.

INTRODUCTION

In many countries, combating desertification is equivalent to promoting development, given the importance of natural resources for the productive system and their degree of deterioration. Although there is no accurate diagnosis of the situation of desertification and land degradation in Latin America, the perception is that such processes are very serious and affect the different ecosystems and countries in various degrees, deepening the situation of inequality and poverty. From 1990 to 2005, Latin America lost 69 million hectares of forests, which represents 7 % of the region's forest cover (1). On this basis, if strict restrictive policies are not applied, a little more than 200 years would be enough to deforest the whole of Latin America.

The FAO Global Forest Resources Assessment (FRA) reported a net loss of forest area of 190.000 ha year for Mexico between 1990 and 2000; 136.000 ha year between 2000 and 2010; a loss of 92.000 ha year between 2010 and 2015; and finally, a deforestation rate of 127.770 ha year for the period 2015-2020 (2).

A typical forest ecosystem contains four layers: trees, shrubs, grasses and microsubstrate. The disappearance of one of them disrupts the entire system, altering the relationships between flora and fauna through the loss of species, which in the long term results in damage to the soil, vegetation and even crops established in the areA. The part elimination of the native vegetation cover also reduces the possibilities of water collection and water vapor production; thus, the aridity of soil increases, rainfall in the area decreases and desertification increases (3). That is why reforestation is a priority activity, not only in Mexico but also in all Latin America, and according to (4) the genus *Acacia* L. (currently known as *Vachellia* L.) is one of the most planted due to its large number of species.

The genus *Vachellia* L. has approximately 1300 species, widely distributed in the tropics of the world. In Mexico, about 84 native species are recognized, of which 30 are endemic, and most are located in arid and semi-arid regions of the country (5).

The most frequent uses found for the species of the genus are: forage, medicinal, fuel, haberdashery and timber. From 30 endemic species in Mexico, 11 are in the category of least concern, 10 in the vulnerable category, 6 in the near threatened category, 2 with different data and 1 as a threatened species (5). In the arid and semi-arid zones of the country, forest resources such as mesquite (*Prosopis* sp.), huizache (*Vachellia* sp.) and ahuehuete (*Taxodium mucronatum* C. Lawson 1851) are of great importance, due to the variety of uses they are put to, whether for exploitation and obtaining wood, charcoal, handicrafts or for feeding small grazing ruminants (6).

The different species of *Vachellia* spp. generally present problems of seed germination under natural conditions, since they have a too hard and impermeable cover that prevents the passage of water (7), so biotechnology is a powerful tool to solve this problem.

Tissue culture of tree species offers a rapid means to produce clonal planting material for the production of woody biomass, reforestation and the creation of germplasm banks for conservation. In general, woody plants are difficult to regenerate *in vitro*, but successes have been achieved specifically in trees of the *Fabaceae* family such as *Dalbergia latifolia* Roxb (8), *Acacia mangium* (9,10). There are several successful reports on the development of seedlings from callus obtained from seedling buds, cotyledon buds and nodal explants (8,11,12). Micropropagation of different Acacia species has been reported by several researchers (13-16).

In vitro culture ensures that a large number of explants are available in minimal space and short time (17).

Plant cells (meristematic) are totipotent, which means that, under suitable conditions, they will give rise to a complete plant with the characteristics of the mother plant. The advantages of this technology with respect to conventional agriculture are: it ensures a continuous supply of plants with uniform quality and yield; rapid production in a short time; it is not affected by seasonal and geographical variations, nor by environmental factors; it is possible to produce new compounds that are not normally found in the mother plant; it is independent of political interference (18).

For all these reasons, the objective of this review is to review the most relevant results on biotechnology, specifically plant tissue culture in species of the genus *Vachellia* L. as trees with potential for the reforestation of desert zones (arid and/or semi-arid) in Mexico.

El cultivo *in vitro* asegura que se cuente con una gran cantidad de explantes en mínimos espacios y poco tiempo (17).

Vachellia spp., general information, uses and applications

The *Fabaceae* family is widely distributed in various regions of the planet, has approximately 500 genera and about 17 000 species, is characterized by its pod-shaped fruits (legume), which may or may not release seeds (dehiscent or indehiscent), presents a diversity of life forms: trees, shrubs, herbs, creeping or climbing plants; it includes a large number of useful plants, especially in terms of food and wood (19).

Within this family there is the subfamily Mimosoideae, composed of more than 1300 species, which are naturally distributed in all continents, with the exception of Europe. The genus *Vachellia* L. is the second most numerous in the family (after *Astragalus*), with approximately 1200 species of shrubs and trees, widely distributed in the tropics of the world. It is the only genus within the tribe *Acacieae* and has about 890 species in Australia and approximately 200 in the Americas (20).

Most of the plants from the Fabaceae family are considered multipurpose species, since they can be used to extract various products such as leaves for fodder and firewood from their stems. In addition, they are widely appreciated for their seeds and pods for livestock feed, and the flowers are used to make perfumes (21).

Mezquite and Huizache are trees that can be found in the hot and semi-desert areas of Mexico. Both trees belong to the same family, so they fix nitrogen in the soil and to do so they use bacteria called rhizobia, which form small nodules on roots. The bacteria take nitrogen from the atmosphere and transform it so that the plants can use it. Nitrogen is one of the main nutrients needed by all plants. These trees play an important role in their ecosystem, have a use in daily life and are a rich source of protein, which was previously consumed in various regions of the country (22).

Among the applications of the genus is the use of the foliage of legumes as feed for cattle and goats in arid regions of Mexico, which constitutes a little studied alternative in ruminant feeding. In particular, huizache pods are characterized by their high nutritional value and could be an ideal material to be used as a source of nutrients for small ruminants (23). Other uses include windbreak species; reforestation of dry forests and degraded grassland areas; for the stabilization of mobile sands in degraded semi-arid regions (24). In addition, the scent obtained from the flowers, known as "cassie", is held in high esteem (25), which has led to the cultivation of species of the genus in southern France and other parts of the Mediterranean (26).

The species of the genus *Vachellia* L. endemic to Mexico and their risk category are shown in Table 1.

From approximately 85 species of *Vachellia* present in Mexico, 35 of them are distributed in the northern Border States, 12 in Coahuila (27), 12 in Nuevo León (28) and 11 in central Chihuahua (29). Most of the species are located in plain areas of arid climates and are an important part of the scrublands in northern Mexico.

Table 1. Species of the genus *Vachellia* endemic to Mexico (5)

Scientific name	Uses	Risk category
Acacia amabilis	Resin use	Threatened species
Acacia anisophylla	Resin extraction	Vulnerable
Acacia biaciculata Vulnerable		Vulnerable
Acacia bilimekii	Forage, fuel, medicinal and in the tannery.	Least concern
Acacia brandegeana	Forage	Least concern
Acacia californicasubsp. californica		Least concern
Acacia californica subsp. pringlei.	Fuel, medicinal (veterinary), and in the construction of houses	Least concern
Acacia cedilloi	Melliferous	Vulnerable
Acacia compacta		Vulnerable
Acacia fusicarpa	With potential for resin extraction	Vulnerable
Acacia gaumeri	Fuel, timber and tranquilizers	Least concern
Acacia glandulifera		Vulnerable
Acacia interior		Vulnerable
Acacia janzenii		Data Deficient
Acacia kelloggiana		Near threatened
Acacia mammifera		Least concern
Acacia mirandae		Near threatened
Acacia occidentalis	Timber	Near threatened
Acacia pacensis		Near threatened
Acacia parviflora	Resin production, as a phytoremediation plant	Least concern
Acacia peninsularis		Near threatened
Acacia purpusii		Vulnerable
Acacia reniformis	Timber, fuel, as potential use for resin production	Least concern
Acacia russelliana		Vulnerable
Acacia saltilloensis		Least concern
Acacia serícea		Vulnerable
Acacia sororia	Potential for resin extraction	Data Deficient
Acacia subangulata	Fuel and tannin extraction	Near threatened
Acacia villaregalis		Least concern
Acacia willardiana	Ornamental plant	Least concern

The exploitation of huizache has caused a decrease in its population in the ecosystem, which causes a significant imbalance, since it is a refuge and food source for other species, which will have to migrate to different habitats due to the processes of survival or extinction (30).

In the arid and semi-arid regions of Mexico, huizache and mesquite are used for reforestation purposes, because they are plants that nourish the soil (by fixing atmospheric nitrogen) and provide shade and shelter for various animals, the wood can be used for construction or as fuel, and they attract insects for pollination (22).

The species of the genus *Vachellia* L. in desert ecosystems are also a source of food for goats and under their branches a characteristic microenvironment is created, which positively influences the quantity and variety of birds, mammals and other plants with which it forms associations such as *Agave* sp., *Yucca* sp. and kakanapo cactus (*Opuntia lindheimeri* Engelm) (31).

Summarizing the above, several species of the genus *Vachellia* L. as for example huizache can be used for reforestation purposes because they are valuable species of which all their parts can be used for multiple purposes: The foliage and pods are useful for livestock feed being used as fodder, especially in dry areas, and also provides them with shade; many birds feed on seeds; the flowers provide essential oils for highly appreciated perfumes; the green pods produce a sticky substance that is used as glue and the trunk provides a gum that is used as a substitute for gum Arabic and is beginning to be commercialized; the bark and pods are useful for dyeing and for obtaining tannins; the wood has multiple applications including poles, furniture, tools and firewood; the tree serves as a living fence and as an ornamental aromatic species. In addition, when used as ground cover in areas with water and nutrient shortages, it controls erosion and provides nesting cover for wildlife. At the same time, its species fix atmospheric nitrogen which favors soil nutrition and increases organic matter when it drops its leaves, thus improving soil structure and subsequent water infiltration.

Reforestation of arid and semi-arid zones in Mexico

Since 2000, a large part of the Mexican territory had already lost its original vegetation cover and large areas had degraded soils due to erosion processes (wind and water), flooding, salinization and contamination. In addition, because of the accelerated degradation, these soils have scarce vegetation, low productivity and are not in the process of recovering their original vegetation cover, making ecological restoration a necessity to rehabilitate them and increase their productivity (32).

The diversity of plant species in arid zones is moderate (6,000 described species) (33) and these have a great potential for natural resources classified as forests, which can be used in a sustainable manner, not only to improve the living standards of the rural sector, due to the alternatives they offer, but also to reforest these areas. This measure would improve soils, quality of life and even water availability, guaranteeing a rational use of these plant resources to avoid activities such as indiscriminate logging.

The northern part of Chihuahua state is characterized by a desert ecosystem composed of a variety of plant associations, including xerophytic and microphilous species (34), which grow in conditions of intense sunlight, scarce precipitation, and nutrient-poor soils. As described above, there are three types of scrubland in desert vegetation: medium subinermeous scrubland on limestone rocks, crassicaule desert scrubland linked to rocks and soils of igneous nature, and microphilous desert scrubland on alluvial soils (35).

In Mexico, due to the high investment needed for reforestation, forest land owners refuse to plant species that could have very slow growth rates or suffer high mortality rates, which is why studies on the behavior of native species used in reforestations are necessary, which, in addition to providing greater security against climate adversities, have a high survival rate, which reduces the inputs to ensure their maintenance and development (36,37). Research has shown that the use of native species in the restoration of disturbed areas can achieve up to 95 % survival (38).

Arid zones have a lower richness of plant species than humid and tropical zones, although in the arid and semi-arid regions of northern Mexico there is a vast and characteristic flora with specialized growth forms (39). For this reason, high priority should be given to maintaining biodiversity in these areas, because the loss of a species in an arid zone means a higher percentage of biodiversity loss compared to other regions with greater species richness (40).

Some research on ecology shows that native shrub species help to halt ecosystem depletion in these environments (41). On the one hand, leaf litter and shrub foliage decrease the speed of raindrops impacting the soil, and on the other hand, roots and trunks reduce the capacity of different climatic agents to transport materials (42).

At present, desertification is a complex phenomenon, conditioned by climatic and socioeconomic events and that there are four main factors in Mexico that accelerate this process: lack of cover vegetation, soil type, climate and anthropogenic disruptors. Reforestation is one of the immediate measures to stop desertification, as well as the damage to soils without vegetation cover; having trees that protect soils and also provide shade and food for animals is essential to preserve life on the planet.

Vegetable tissue culture in huizache (*Vachellia* spp.)

Vachellia spp. are among the most widely planted forest species worldwide, and the most widely used in the recovery of degraded areas (43). Currently, it is playing an increasingly important role in efforts to sustain the commercial supply of forest products and, at the same time, reduce the pressure on natural forest ecosystems, for all the utilities described in the previous section.

The asexual propagation of *Vachellia* has been studied; however, many difficulties persist, which represents a limitation for the implementation of precision silviculture in the cultivation of this species. The sprouting of decapitated trees, even in juvenile stages, is difficult and it is not frequent to find in nature juvenile stages as a source of material for vegetative propagation (establishment of clones), so micropropagation has been used as a mechanism to massively multiply clones of forest species of high economic value (44).

Generally, the species of the genus can be reproduced by seed (45), but as explained above, they present germination problems under natural conditions, due to the too hard and impermeable cover that prevents the passage of water (7). Seeds can be sown in seedbeds and transplanted 6 to 10 days after sowing, however, by this method only 37 % seedlings are recovered (46). For this reason, micropropagation plays an essential role in obtaining seedlings of these species, and thus being able to provide forest nurseries with sufficient plant material to reforest desert areas.

Micropropagation can be defined as the technique in which any part of a plant (roots, stem, leaves, seeds or protoplasts), cultivated in an artificial nutrient medium, under aseptic conditions, gives rise to a complete plant (47,48). The first idea of growing an individual plant in an artificial medium came from Haberlandt in 1902, although he never realized the relevance of his approach, but more than 100 years later, this technology remains an essential tool for plant sciences (47). Tissue culture is used for an increasing number of purposes, such as crop improvement programs, embryo rescue, production of haploids and dihaploids in a short time, conservation and rescue of endangered species (39,49).

Some phases for plant regeneration from isolated explants during in vitro culture are (50):

Algunas fases para la regeneración de plantas, a partir de explantes aislados, durante el cultivo *in vitro* son (50):

- Selection of the plant, seed or plant tissue;
- Establishment: disinfection of explants, generally with sodium hypochlorite (NaOCl) and their subsequent adaptation to the artificial culture medium to obtain callus, sprouts or somatic embryo, as desired;
- Multiplication: obtaining sufficient plant explants for the regeneration of the number of plants required;
- Rooting: formation of roots in plant explants;
- Acclimatization of seedlings obtained *in vitro* to *ex vitro* environmental conditions (soil or some inert substrate).

There is very little *in vitro* work on huizache. Work has been done on the in vitro propagation of *Acacia mangium* Willd (51), since it is a forest species used for its wood quality and rapid growth. However, the same author mentions that clonal propagation studies are very few. Disinfection was performed with different concentrations of NaOCl (0.5; 1.0 and 1.5 %) and antibiotics (Cephalexin, Amoxicillin and Ambramycin, 2 mg mL^{-1}) and they were established in MS culture media (52), with different concentrations (0; 0.44; 0.88 and 2.22 μM) of 6 BAP (6 Benzylaminopurine). The data showed that 1.0 % NaOCl and cephalexin (2 mg L^{-1}) allowed obtaining 67 % of explants free of contamination. The highest average number of shoots was obtained with MS medium supplemented with 6 BAP at 2.22 μM.

For *A farnesiana*, seeds were used that were germinated on 100 % MS medium without plant growth regulators (PGR), on cotton saturated with sterile distilled water and in commercial soil, and showed 100 % germination after 2 days. Cotyledons were used as explants and grown on MS medium supplemented with different concentrations and combinations of PGR (6 BAP 0, 4.4 and 6.7 μM, or 6 BAP and 2,4-dichlorophenoxyacetic acid (2, 4-D): 4.4 and 4.5 μM, respectively). The highest number of multiple sprouts was on MS medium with 4.4 μM of 6 BAP with 5.42 ± 0.98 shoots per explant and rooting was achieved with 17.12 μM of AIA (indolacetic acid) (53).

The highest number of sprouts was obtained when using BA (benzyladenine) 4.0 μM, a concentration much higher than kinetin (Kin) to cause multiplication of *A. mangium* (54). The superior effect of BA on Kin in organogenesis *in vitro* has been reported in many Acacia species (15,55,56). Interestingly, supplementing MS medium with Kin (1 mg L^{-1}) did not result in direct regeneration of *A. nilotica*, proliferation remained undifferentiated for a month, until ANA (naphthaleneacetic acid) (0.6 mg L^{-1}) was used, which in turn induced sprouts multiplication almost instantaneously (57).

Several investigations report that the incorporation of auxin together with BA to the culture medium improves the frequency of differentiation of buds and shoots instead of using only BA, specifically 2.0 lM BA plus 1.0 lM NAA was a favorable combination for direct organogenesis of *A. mangium* sprouts (54). In a similar study in *A. auriculiformis*, the MS medium was supplemented with 2 mg L^{-1} Kin and 0.5 mg L^{-1} NAA and exhibited a maximum frequency of shoot regeneration (58). For the species *A. ehrenbergiana* (59) they used cotyledons grown on MS culture medium and 90.3 % of explants showed regeneration with a BA supplement (10 lM) and the maximum number of sprouts (7.3) were obtained on culture medium supplemented with BA and NAA (0.1 lM). Therefore, in these species, the combination of cytokinins and auxins is relevant for efficient sprout regeneration.

Some researchers worked on micropropagation of *A. mangium* from seeds or from explants collected from outside and the process was initiated in MS basal culture medium supplemented with 4.4 μM BA (60). Cultures of microsprouts produced by axillary budding were grown and maintained by regular subcultures every 60 days in fresh MS culture medium to which 2.2 μM BA + 0.1 μM ANA was added. This procedure improved organogenic capacity for shoot multiplication by axillary budding, with average multiplication rates of 3-5 every 2 months.

For propagation of *A. farnesiana* from multiplication of nodal explants derived from *in vitro* grown plants, MS medium supplemented with BA and Kin at different concentrations was used (56). The concentrations used for shoot initiation were: of BA (0.5; 1.0; 1.5; 2.0; 2.5 mg L^{-1}) and Kin (0.5; 1.0; 1.5; 2.0; 2.5 mg L^{-1}). The highest number of sprouts was 75 % in the medium with BA, with an average size of 8.5 cm, while when explants were grown in Kin, only 55 % of them proliferated. After four weeks the excised sprouts (1-2 cm) were rooted in medium concentration MS supplemented with 0.5 mg L^{-1} of IBA (indolbutyric acid) and 0.05 mg L^{-1} of NAA, resulting in 90 % of rooted seedlings.

In *A. nilotica,* an author performed micropropagation from nodal segments in MS culture medium supplemented with 2.5 mg L^{-1} of BA, obtaining the highest average number of sprouts per explant (3.58). In *A. Senegal* (62) an author reported that zeatin, which is a natural cytokinin, produces better induction of multiple sprouts.

Research was published addressing somatic embryogenesis for the procurement of *Vachellia* spp. seedlings *in vitro* where immature zygotic embryos of *A. farnesiana* and in a semisolid MS basal culture medium supplemented with 2,4-D 9.05 µM and Kin 4.65 µM to induce callus and somatic embryos were produced in semisolid differentiation media without growth regulators or with abscisic acid (ABA) reporting that the addition of ABA increased the percentage of embryos that reached more advanced differentiation stages (63).

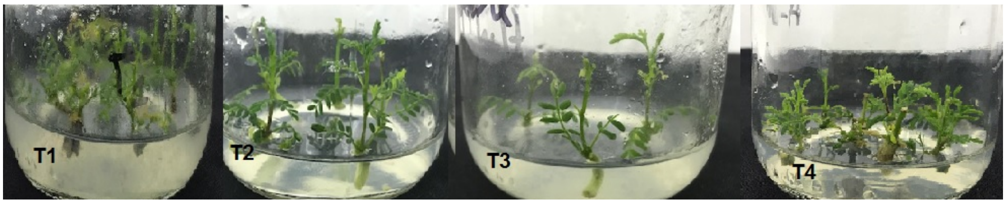
Regarding the acclimatization phase, some researchers have successfully adapted *in vitro* seedlings of Vachellia spp. to *ex vitro* conditions using different substrates and adaptation conditions, achieving survival rates of more than 60 %. *A. chundra* seedlings were transferred to sterile sandy soil in a 1:1 ratio (garden soil: sand) in clay pots and maintained under high humidity (70 %) in a greenhouse with a temperature of 30 ± 2 °C obtaining 65 % survival (15). For the species *A. ehrenbergiana* (59) they used sterile soil (Keltech Pvt Ltd., Bangalore) in plastic pots to acclimatize *in vitro* rooted seedlings and maintained them at 24 ± 2 ℃ and with a photoperiod of 16 h of light by watering every third day with half concentration of MS saline solution for 2 weeks, after 4 weeks, acclimatized plants (80 %) were transferred to pots containing normal garden soil and kept in greenhouse with natural light until transferred to the field. On grated coconut husk substrate, a 90 % survival rate of *A. auriculiformis* seedlings was obtained during acclimatization in the greenhouse with a temperature of 25 to 30 °C and relative humidity of approximately 80 % with 50 % shade (16). After rooting, (12) carefully removed the explants from culture tubes and transplanted them directly into pots on vermiculite substrate mixed with river sand (1:1) and 80 % of the seedlings survived. Similar survival rates were obtained by (53) using a commercial soil (Nutrigarden®) on *A. farnesiana* and *Prosopis laevigata* seedlings.

In this subject, some of the authors of this review (64) worked with seeds of *Vachellia* sp. collected in the Northern Urban Zone of Chihuahua City in 2019 and used three scarification treatments; T1: Immersion in hot water (H_2O) for 5 minutes (physical scarification); T2: immersion in concentrated sulfuric acid (H_2SO_4) for 20 min (chemical scarification); T3: immersion in concentrated hydrochloric acid (HCL) for 20 min (chemical scarification) and after disinfection (2 % sodium hypochlorite for 20 min) they seeded them on Agar-Water medium (6. 5 g L^{-1}) for germination. For multiplication of seedlings *in vitro* they used MS culture medium (1962) supplemented with sucrose 30 g L^{-1}, phytagel 2.5 g L^{-1}, activated charcoal 2.5 g L^{-1} and different growth regulators (T1: 6 BAP 0.5 mg L^{-1}, Kin 0.5 mg L^{-1}, Chitosan 60 mg L^{-1}; T2: 6 BAP 1 mg L-1, Kin 0.5 mg L-1, Chitosan 60 mg L-1; T3: 6 BAP 0.5 mg L-1, Kin 0.5 mg L^{-1}; T4: 6 BAP 1 mg L^{-1}, Kin 0.5 mg L^{-1}). As a result of the scarification used, approximately 35 % seed germination was obtained with T2 (H_2SO_4) for 20 min (Figure 1) and in the multiplication phase the explants had better growth and multiplication percentage in T2 (Figure 2). In this case the combination of two cytokinins was essential to obtain the desired results with a multiplication coefficient of 3 while in some of the research presented the authors combine auxins and cytokinins. These combinations depend largely on the objective of the research and the species.

Several species of *Vachellia* spp. have received due importance in tree tissue culture because of their proven ability to reclaim wastelands and their ecological and economic importance. Techniques based on cell, tissue and plant organ cultures have been employed in forest tree research for successful reforestation and forest management programs. The relevance of tissue culture methods has gained momentum to meet the growing demands for biomass and forest products.

A: T1 (immersion in H_2O for 20 min), B: T2 (concentrated H_2SO_4 for 20 min) and C: T3 (immersion in concentrated HCL for 20 min)

Figure 1. Viability and germination of the *in vitro* culture of huizache (*Vachellia* sp.)



T1- 6 BAP 0.5 mg L^{-1} + Kin 0.5 mg L^{-1} + Chitosan 60 mg L^{-1}; T2- 6 BAP 1 mg L^{-1} + Kin 0.5 mg L^{-1} + Chitosan 60 mg L^{-1}; T3- 6 BAP 0.5 mg L^{-1} + Kin 0.5 mg L^{-1}; T4- 6 BAP 1 mg L^{-1} + Kin 0.5 mg L^{-1}

Figure 2. Explants of *Vachellia* sp. in the different treatments used in the multiplication phase of (First subculture)

Several methodologies for micropropagation of species of *Vachellia L* genus have been achieved, including the adaptation of seedlings to *ex vitro* conditions, but there is very little information regarding the species *Vachellia farnesiana* and in general in all cases (all species of the genus) research is not recent. In the genus under study, there is no information on haploid production and only one study has dealt with somatic embryos. The most researched topics are direct organogenesis as shown in this section.

CONCLUSIONS

- Nowadays, due to climate change and the irrational use of finite resources such as water and soils, reforestation is becoming increasingly necessary and forest species are of great utility, not only for these purposes, but also for the release of dioxygen into the atmosphere.
- The currently available literature on plant tissue culture of species *Vachellia* L. genus shows complete methodologies for very few species and the vast majority are not from the last five years.
- A competent plant tissue culture protocol should have all the necessary steps to get the seedlings to the nurseries and then transported to the areas to be reforested.

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