

Chapter

Genetic Engineering of Purslane (*Portulaca oleracea* L.)

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Abstract

Portulaca oleracea L., popularly known as purslane, is an herbaceous succulent plant classified as one of the most important invasive weeds in the world. Due to its high nutritional level and wide range of pharmacological effects, involving anti-inflammatory, antibacterial, antioxidant, and antiulcerogenic, purslane is one of the medicinal species listed by the World Health Organization. In addition, purslane produces several phytochemicals, including flavonoids, alkaloids, and terpenoids, which confer different pharmacological activities and make the plant highly attractive for use in the most diverse industries. It has high adaptability to extreme soil conditions, able to grow and spread in environments under drought stress, salinity, and poor nutrients; and has been presented as a potential model plant to study resistance to abiotic stresses. Among other purslane traits of interest to the agriculture sector, is worth to mention phytoremediation and allelopathy, thus being a sustainable alternative in organic agriculture. Here, we report a bibliometric analysis of purslane *in vitro* tissue culture and genetic modification/editing, and discuss opportunities and limitations to exploit the biotechnological potential of purslane as a source of valuable bio-molecules for many different industries.

Keywords: purslane, medicinal plant, multipurpose species, genetic transformation, tissue culture, biolistic, agrobacterium, abiotic stresses

1. Introduction

Portulaca oleracea L. (**Figure 1**), the most well-known species of the *Portulaca* genus, is commonly known as purslane, or common purslane, according to Ref. [1]. This genus belongs to the family Portulacaceae, order Caryophyllales, superorder Caryophyllanae, class Magnoliopsida, subdivision Spermatophytina, division Tracheophyta, superdivision Embryophyta, infrakingdom Streptophyta, and subkingdom Viridiplantae [2, 3].

Purslane is classified as a multipurpose plant species [4]. Plants cultivated for the purpose of providing more than one significant contribution to the production



Figure 1.
Purslane (*Portulaca oleracea* L.).

and/or service functions of a land use system are defined as multipurpose plants. They are classified according to the attributes of the plant species and the functional role of it in the technology under consideration, be it linked to the agricultural, pharmaceutical, chemical, or other economic sector.

2. Socioeconomic importance of purslane

2.1 A medicinal plant recommended by the World Health Organization

According to the World Health Organization (WHO), purslane is one of the most used medicinal plants. Known as a “Global Panacea”—a remedy supposed to heal all sicknesses, it is used extensively in folk medicine due to its wide array of health effects [5, 6]. The ethnobotanical importance of purslane led to various studies confirming its pharmacological properties. Those studies support its use as an antibacterial [7], anti-inflammatory, antioxidant [8], neuro- and hepatoprotective [6], antidiabetic [9], and antiulcerogenic agent [10], among other applications. In addition, it is reportedly a highly nutritious plant, being among the top terrestrial sources of essential fatty acids, tocopherol, ascorbic acid, glutathione, and other components, which suggests its nutraceutical potential [11, 12]. These valuable chemical constituents result from purslane’s diverse set of chemical pathways.

All organisms have an integrated network of chemical reactions meticulously mediated and regulated by enzymes. It encompasses primary and secondary metabolic pathways synthesizing various organic compounds [13]. More specifically, primary metabolism involves generating components required for growth and development. Its products often serve as intermediates for the production of specialized chemicals that comprise the secondary metabolism, which plays a crucial part in a plant’s interaction with the environment [14]. Processes that result in primary metabolites are highly conserved, while those of secondary metabolites are lineage-specific and continuously influenced by abiotic and biotic factors. That results in the formation or suppression of bioactive compounds that confer specific properties to the plant, intending to promote its survival and protection [15, 16].

Following a simple classification, secondary metabolites are divided into three main groups: phenolics, nitrogen-containing compounds, and terpenoids. Each is obtained through different biosynthetic pathways, resulting in chemicals with distinctive structures that confer valuable properties. These are building blocks to

the manufacturing of high-added-value products focusing on health, nutrition, or agriculture, and thus are of enormous importance within the scientific and industrial framework [16, 17]. Purslane has a rich and unique content of these bioactive compounds that, individually or synergistically, provides beneficial effects and explains its extensive use in folk medicine [6, 12]. The following paragraphs will give insight into some highly important secondary metabolite groups and their known activities.

Flavonoids, which comprise the phenolic group, are among the main active ingredients from purslane—with kaempferol, luteolin, apigenin, myricetin, and quercetin as its major components. In addition, novel structures, namely portulacanonones and oleracones, were first isolated from this plant. Studies have shown the anticancer [18, 19], anticholinesterase [20], anti-inflammatory, and antioxidant effects of these flavonoids [21, 22]. Furthermore, families belonging to Portulacaceae produce betalains, known as nitrogen-containing plant pigments with limited occurrence in nature [23, 24]. This subgroup is natural colorants in the food and cosmetic sectors, although studies have shown their neuroprotective [25], chemoprotective [26], and antimicrobial potential [24].

N-trans-feruloyltyramine, dopamine, noradrenaline, and oleraceins are alkaloids also identified in this plant species [27]. These nitrogen-containing compounds have reported immune-enhancing and neuroprotective effects, among others, and underwent studies for the prevention and treatment of neurodegenerative diseases [28–30]. The terpene content, which includes portulosides A-B, portulenes, and others, also contributes to potentializing antimicrobial and hepatoprotective effects of purslane extracts, and so on [27]. Other bioactive components include lignans, phenolic acids, and esters [31], and new molecules are constantly isolated from this plant through various methodologies [30, 32, 33].

Purslane is also a rich source of omega-3 and omega-6 fatty acids, thus contributing to its nutritional value [34]. These are the precursors of eicosapentaenoic and docosahexaenoic acids, which can reduce the risk of cardiovascular and cerebral diseases [12]. Studies on the development of functional food products from this plant are already available [35], as it also has considerable amounts of vitamins and dietary minerals [11]. Overall, each phytoconstituent mentioned contributes to establishing the ethnobotanical importance of purslane and supports the application of this plant in the pharmaceutical, food, and cosmetics industries.

2.2 A source of so many agricultural important traits

Besides being a source of many traits for the pharmaceutical and chemical industries, purslane is also a source of features of direct importance to the agricultural and agri-industrial sectors. Among the most important ones are phytoremediation, allelopathy, and tolerance to biotic and abiotic stress. Below we present some insights into some of those traits and then—more to the end of this chapter—report intensively on resistance to salinity stress.

Due to the purslane tolerance capacity for metal stress, it undergoes phytoremediation and biomonitoring studies in the field and closed conditions [36]. Phytoremediation is an economic process that exploits plants' capacity to accumulate heavy metals in polluted habitats by their harvestable parts [37], while biomonitoring is the capacity to monitor contaminated environments [36]. Mohammadzadeh and Hajiboland [38] reported a successful study using purslane in phytoremediation strategies to remove nitrate from nitrate-contaminated sites.

Allelopathy is the ability of a plant to suppress the germination, growth, survival, and reproduction of other plants in its surroundings. It produces and releases allelochemicals (secondary metabolites) that negatively affect other plants. Hamad [39] showed that aqueous extracts from purslane shoots and roots have allelopathic (inhibitor) effects on seed germination and the growth of monocots and dicots. Rashidi et al. [40] investigated the allelopathic effect of purslane on seed germination and growth of several plant species and demonstrated its allelopathic potential against *Phaseolus vulgaris* L. and *Allium cepa* L. as it reduced their seed germination rate.

After studying the chemical composition and yield of six purslane genotypes, Petropoulos et al. [34] reported that the biomass yield (fresh weight) in the open field was affected by genotype, with the highest yield of the tested genotypes being 33 tons/hectare, and the lowest being 11.5, with an average of about 22.5 among these genotypes. Kong and Zheng [41] evaluated the potential of producing purslane in a hydroponic system by testing two distinct cultivars—Green and Golden. Both cultivars performed similarly, generating a marketable yield of approximately 5.75 kg per m² on a bimonthly basis, which might yield 345 tons/hectare/year if cultivated in a bimestrial regime. Alu'datt et al. [42] evaluated the effect of different soil-less substrates on the fresh yield of purslane over five harvest cycles during the growing season under closed conditions and reported productivity of approximately 27 kg per m² when using Tuff: Peatmoss (2:1) substrates; what might yield 270 tons/hectare/year.

Purslane is a succulent herbaceous halophyte plant classified as invasive and considered the eighth most common weed in the world; it grows in warm moist places during the summer and spring seasons and can grow in almost any unshaded area, including gardens, crop fields, and waste places [43]. Because of that, its outdoor production in extensive areas faces several concerns. However, the above-mentioned high productivity of purslane in the context of controlled-environment agriculture [44–46] can open many doors of opportunities for the purslane industry. Many of those might take advantage of having a highly efficient protocol for engineering/editing purslane genome.

3. Genetic engineering/editing of purslane: state of the art

Genetically modified/edited plants are usually developed by *in vitro* regeneration from single transformed cells, and because of that, using *in vitro* plant tissue culture-based methods is required. However, that is not the only way to develop such types of plants. Some strategies of plant transformation that do not depend on *in vitro* regeneration are available and are known as “*in planta*” transformation methods. The floral dip transformation method is the most well known of them [47]; however, no report is available on its successful use in purslane.

3.1 Purslane *in vitro* tissue culture

Once the goal is the *in vitro* regeneration from single transformed cells, it is necessary to develop first a reliable and efficient purslane tissue culture protocol. Such a process may take advantage of the organogenesis or embryogenesis capability of the plant species in question and need to evaluate some factors such as the most appropriate type of explant, culture medium, growth regulators, and cultivation conditions, among others [48]. Unfortunately, there are not many reports on purslane *in vitro* tissue culture. The few ones available will be reported in the next paragraphs.

Safdari and Kazemitabar [49] was the first report on *in vitro* regeneration of purslane plants, intending to determine the best hormonal treatment for the induction of embryogenic callus from leaf tissue, the best type of explant and hormones for plant regeneration, and root induction from regenerated shoots. Later, Sharma et al. [50] reported an attempt to establish an efficient *in vitro* protocol for plant regeneration through organogenesis, using 1.5 cm long knots as explant, and achieving a stable efficiency of 70%.

Shekhawat et al. [51] reported an efficient *in vitro* regeneration method for purslane using a liquid medium, where the explants used were shoots with one and two nodes, obtaining a rooting efficiency rate of 96%. Oraibi et al. [52] reported success in efficiently inducing callus from purslane leaves, with subsequent production of extracts from the callus that presented antibacterial activity.

Purslane is sexually propagated, producing an enormous amount of seeds in a short period—within 60–90 days. Besides, purslane is also efficiently vegetatively propagated from cutting. The success of propagation (by seeds or cuttings) is probably one of the reasons that justify that there are not many reports on purslane *in vitro* tissue culture. The lack of demands for eradicating pathogens could be another reason to explain it.

The demonstrated capacity for producing over a hundred tons of biomass per hectare per year under closed conditions [42] makes purslane an ideal candidate as the crop to produce its bio-molecules, reducing the risk associated with the fact it is a weed [43]. However, one cannot forget that the growth of purslane cell suspension using bioreactors [53] is another way ahead to produce such bio-molecules under a controlled environment. In such case, there is the need to develop protocols to obtain and maintain purslane cell suspension.

Consequently, there is no doubt that for purslane to become a model plant for functional genomics research, aiming to advance on the exploitation of so many of its bio-molecules—whether in the pharmaceutical sector, in the agronomical sector, or in other sectors—the scientific community must expand and deeper the studies in many of the frontlines of plant tissue culture, such as haploid/di-haploid production, cell suspension production and maintenance, and, of course, genetic modification/editing. The results of the tissue culture survey on *Portulaca oleracea* are summarized in the table below (Table 1).

3.2 Genetic modification of purslane

The genetic transformation of plants involves the insertion, integration, and expression of exogenous genes into the genome of a plant species. One of the main focuses in obtaining transgenic cultures is incorporating new characteristics, studying primary biological processes, and producing bio-pharmaceutical proteins. Since the 1980s, different techniques became available for introducing heterologous genes into the genome, among which the transformation mediated by *Agrobacterium* and biolistics stands out [55]. The sonication-assisted *Agrobacterium*-mediated gene transfer system increases the transformation efficiency [56], and studies using sonication associated with vacuum infiltration proved to be efficient when applied to different cultivars of economic importance [57].

Sedaghati et al. [54] aimed to develop an *Agrobacterium*-mediated transformation and regeneration system using somatic embryogenesis in purslane, obtaining an efficiency of 72.22% from leaf explants. Studies carried out by the same group in 2021, seeking to optimize this transformation process, used sonication associated with

References	Explant	Multiplication technique	Efficiency	Objective
Safdari and Kazemitabar, [49]	Petioles, shoot tips, and leaves.	Callogenesis	70%	Test different hormones and explants.
Sharma et al. [50]	Nodal shoot segments	Organogenesis	70%	Development of an efficient regeneration protocol.
Shekhawat et al. [51].	Nodal shoot segments	Organogenesis	96%	Regeneration in liquid medium and implications of growth regulators.
Oraibi et al. [52]	Leaves	Callogenesis	80%	Antibacterial Activity.
Sedaghati et al. [54]	Seeds and leaves	Somatic embryogenesis	72.22%	Development of an efficient regeneration protocol.

Table 1.

Published findings on in vitro tissue culture of purslane (*Portulaca oleracea* L.) - search done in December 2022.

vacuum infiltration from seeds, obtaining a maximum efficiency of $39.25 \pm 2.88\%$. Subsequently, Sedaghati et al. [58] carried a new study out where they applied purslane as a green bioreactor to evaluate the expression of the HSA human serum albumin gene.

Two reports describe success in transforming purslane using *Agrobacterium rhizogenes* [59, 60]. Tandon et al. [59] in an attempt to improve the phytoremediation of municipal waste water by increasing the root biomass, transformed four distinct plant species using *A. rhizogenes*, including *P. oleracea*. They used nodules and roots as explants for transformation, and achieved an increase in phytoremediation efficiency of transformed portulaca for the treatment of municipal waste water was observed over the non-transformed plants. The study by Ahmadi Moghadam et al. [60] aimed to optimize and evaluate the production of dopamine in hairy roots of purslane, using *rolB* as the gene of interest; and roots, stems, leaves, and cotyledons as explants. It was found that the most suitable explants for the induction of hairy roots were the cotyledons, with an efficiency of 53.3%. The effect of methyl jasmonate and salicylic acid on

References	Explant	Technique	Efficiency	Gene
Tandon et al. [59]	Nodal segments and roots	<i>Agrobacterium rhizogenes</i>	42–68%	–
Moghadam et al. [60]	Cotyledon leaves	<i>Agrobacterium rhizogenes</i>	53.30%	<i>rolB</i>
Sedaghati et al. [54]	Stem and leaf explants	<i>Agrobacterium</i> -mediated	72.22%	<i>GUS</i>
Sedaghati et al. [57]	Seeds	<i>Agrobacterium</i> -mediated transformation using sonication and vacuum infiltration.	39.25%	<i>uidA</i>
Sedaghati et al. [58]	Seeds and leaves	<i>Agrobacterium</i> -mediated transformation.	100%	HSA

Table 2.

Published findings on genetic modification in purslane (*Portulaca oleracea* L.) - search done in December 2022.

the accumulation and biosynthesis of dopamine in hairy roots culture of purslane was investigated, showing that elicitation with the former resulted in about 4.3-fold higher dopamine yield compared to the control hairy root cultures. The results of the genetic modification survey on *Portulaca oleracea* are summarized in the table above (**Table 2**).

4. Purslane as a model plant to study resistance to abiotic stresses

Purslane is a vegetable highly adaptable to various climates and adverse conditions (such as heat, drought, and salinity), giving it a competitive advantage over many other plant species produced [48]. It can grow in arid and saline soils, and it is considered both a halophyte (adapted to salty environments) and a xerophyte (adapted to dry ones); it is also listed as a halophyte plant in the eHaloph database [3, 61, 62].

Ren et al. [63] characterized the responses of 10 different purslane accessions to drought stress, and two African accessions were the most drought tolerant ones, Tokombiya and Egyptum. Drought tolerance was highly variable among the tested accessions at seed germination, seedling development, and adult stages. Jin et al. [64] evaluated the effect of drought and heat stress on purslane, individually and simultaneously. They demonstrated different survival strategies to physiological and metabolic stress, expressed as an increase in malondialdehyde, electrolyte leakage, O₂ and superoxide dismutase, and peroxidase activities, and a decrease in chlorophyll content. Furthermore, they suggested that combined stresses have a more severe effect on purslane plants compared to individual stresses. Another indication is that purslane is a promising candidate to be used in the recovery of ecosystems in arid and semi-arid regions [64].

Another very important fact about purslane is that, depending on the environmental condition, it can switch between the C4 photosystem and Crassulacean acid metabolism (CAM). Ferrari et al. [65] used purslane as a model system to investigate the involvement of the circadian clock, transcription factors, and plant hormones in coordinating the expression of C4 and CAM genes. They showed that, in general, the endogenous circadian clock coordinates and optimizes the daily time of regulation of the C4 and CAM genes in purslane leaves that are well-irrigated and under water stress [65].

There are studies of taxonomy, ecology, physiology, biochemistry, and genetics of purslane, including those characterizing its genetic variability. That information already available regarding the development of purslane, together with characteristics such as a short life cycle, small size, and relatively easy handle, reinforces the idea of turning purslane a research model plant for a better understanding of plant resistance to those two abiotic stresses [66, 67]. It is necessary to use different model plants, instead of the regular ones—*Arabidopsis*, for instance—as responses to those stresses are different between species [68].

Our research group developed a robust salt stress protocol (**Figure 2**) to characterize the morphophysiological responses of young purslane plants to salinity stress, along with multi-omics analyses, where it was possible to observe three distinct levels of salt stress by electrical conductivity gradients and water potential in substrate saturation extract [69]. The multi-omics integration studies showed 51 pathways having at least one enzyme and one metabolite differentially expressed in the leaves of purslane as a result of salt stress [69]. The characterization of the metabolomics, transcriptomics, and proteomics profiles on the leaves and roots of adult purslane plants were generated, and their analyses allowed further insights on the resistance of purslane to salinity stress (Rodrigues-Neto et al., unpublished).



Figure 2.

Purslane (Portulaca oleracea L.) submitted to salinity stress studies at Embrapa Agroenergia during seed germination, seedling development, and different adult stages. Source: Souza Jr., M. T.

Salinity tolerance is an important agronomic feature due to the great problem of salinized soil, which corresponds to 20% of the irrigated land in the world [48]. Studies carried out by Hamidov et al. [70] suggest the use of purslane to promote the rehabilitation of saline soils in the northern part of Uzbekistan, in addition to highlighting its great nutritional quality and its efficiency in tolerating chloride salinity, which makes this species a potential candidate for biosaline agriculture [70].

Yang et al. [30] applied physiological and comparative proteomics to investigate the mechanisms underlying purslane's tolerance to high-temperature and high-humidity stresses, demonstrating that this plant species deploys multiple strategies to cope with these combined stressors. Several strategies, from the activation of several metabolisms to the transient development of a CAM-like metabolism, were responsible for increased adaptation to water stress and combined stress in purslane [71].

To become a reliable model plant in functional genomics studies aiming to characterize the genetic basis of the resistances to those different abiotic stresses, it would be helpful to avail a reference whole genome sequence, as well as an efficient and reliable genetic modification/editing protocol.

5. Purslane as a source of bio-molecules of biotechnological importance

5.1 Screening for bio-molecules in purslane biomass

The final composition of bioactive compounds in purslane is affected by harvesting period, soil characteristics, cultivation practices, biotic/abiotic stresses, and other

environmental factors, besides the genetic background. Temperature conditions during storage also play an essential role in this plant's quality, as it may lead to the degradation of particular components. Moreover, the efficiency of the extraction process is highly dependent on the plant's material, target metabolites, the temperature set, and the type of solvent used. Understanding these aspects that result in purslane's and its extract variability can allow for the management of specialized chemicals, thus leading to increased added-value products [12, 34, 72].

Novel bioactive compounds are continuously being isolated and identified from purslane through different extraction methods, including alkaloids [32, 73], flavonoids [22, 31], lignans [31, 74], and so on. Furthermore, changes in the metabolism—caused by biotic and abiotic factors—can be analyzed through various analytical methods. Metabolomic profiling techniques can determine and compare the chemical content of cultivars grown in varying conditions [12]. For instance, studies have shown that certain levels of salinity treatment result in increased concentration of valuable metabolites in purslane. Salt, as abiotic stress, triggers the plant to potentialize specific chemical pathways. However, it is essential to consider that these effects may also depend on the cultivar and geographic distribution, among other factors. Still, this indicates the potential for purslane's accessions to be explored for stress-induced augmented production of bioactive compounds [12, 48].

Moreover, fermentation can enrich the profile of active compounds found in purslane. During this specific process, enzymes derived from the metabolism of microorganisms promote the degradation of antinutritional factors and organic complexes. That gives rise to low-molecular-weight compounds—such as free amino acids [75] that can serve as substrates in the biosynthetic routes of secondary metabolites [76]. Lactic acid fermentation, for instance, is a suitable tool that explores the functional potential of purslane's biomass. It promoted increased bio-availability and profile modification of its specialized constituents and supplemented the matrix with active metabolites from the bacteria. Based on this, choosing a microbial group with high metabolic potential and versatility could lead to a selection of suitable strains for effective biotechnological processes [77, 78].

In summary, purslane is a rich source of specialized chemicals, and abiotic and biotic factors strongly influence their final content. Post-harvest treatments and extraction methods may also result in compound variability. Based on that, this species is the source of novel phytochemicals, and many techniques are employed to understand those aspects and compare cultivars. Stress-induced methods can lead to obtaining higher concentrations of bioactive compounds. Additionally, fermentation and biotechnological processes are promising strategies for exploring its functional potential.

5.2 Bio-molecules to control plant pathogens

The intensification of agricultural activity through the implementation of extensive areas of monoculture led to the emergence of pests at high levels, capable of reaching the threshold of economic damage. Groups of pathogens such as fungi, bacteria, viruses, nematodes, and insects are biotic agents whose infection to cultivated plants drastically reduces productivity. In the case of phytonematodes, global losses reach values above US\$ 100 billion annually [79], with emphasis on the species *Meloidogyne incognita*. Several methods can be used to control phytopathogens, and the integration of these different strategies results in the most effective form of management. However, under high population densities, the suppression of these

pathogens is largely carried out by making use of synthetic chemical pesticides, products that represent a risk of intoxication for humans and animals, as well as contamination of the environment. Chemical pest control substantially burdens the production process and also incurs the risk of selecting resistant organisms [80]. In view of all the problems involved in the adoption of this control method, it is necessary to search for more sustainable measures as an alternative way to contain the attack of agricultural pests. A trend that has shown promise and is gaining space in the field of research is the investigation of the activity of botanical extracts against plant parasites [81].

A vast number of species belonging to different botanical families can produce, through their secondary metabolism, several compounds from different chemical classes with the potential to suppress pest attacks. Chemical groups, such as alkaloids, terpenes, coumarins, flavonoids, among others, represent some of the elements responsible for inhibiting the action of various phytoparasites and, therefore, have competence to serve as a basis for the development of more eco-friendly pesticides, and mitigate the negative effects caused by pesticides [82]. Brazil has a mega diversity of plant resources with potential to be exploited in the prospection of biopesticides [83].

Botanical extracts can be produced from different structures, such as roots, flowers, seeds, and leaves, among others, and even from industrial co-products using different extracting solutions [84]. In this context, the use of these solutions with different physical-chemical characteristics allows obtaining a greater number of compounds, of different chemical classes with multiple applicability related to the medical, cosmetics, livestock, and agricultural areas [85]. Purslane is a plant of wide geographic distribution and highly attractive as an object of study for having a rich variety of phytochemicals with biocidal action. Furthermore, the use of plant residues as a green technology in pest control is a bi-sustainable possibility. This measure is characterized by the removal of materials from the industrialization process, for the production of sustainable pesticides, replacing conventional agrochemicals, whose molecules are generally classified as having a high toxicological degree [86].

Among the advantages of using phytochemicals in pest management is the possibility of associating extracts from different species, aiming at obtaining multifunctional technologies without generating incompatibility between the different molecules that participate in the compounds, which commonly happens in mixtures of synthetic chemicals [87]. Besides, the miscellany of extracts from different botanical families might result in the development of technologies that control two or more pathogens simultaneously, without negatively affecting the community of beneficial microorganisms in the soil.

Identified and characterized botanical compounds with biocidal effect can be used as tools in activities devoted to the discovery of genes and gene products involved in their synthesis routes. Such information is later used in metabolic engineering studies, which will allow the synthesis of the compound of interest in different parts of the plant, such as the root, for the control of soil pathogens. In addition, extracts and botanical compounds can also be used as basic constituents in the generation of green nanoparticles [88, 89]. Brazil has one of the greatest biodiversity on the planet and represents a relevant source of natural plant-based products. This fraction of biodiversity constitutes the so-called plant genetic resources. At the Brazilian Agricultural Research Corporation—Embrapa, a significant inter- and intraspecific genetic variability is conserved in active Germplasm Banks. These banks represent a source of diverse materials with numerous physicochemical characteristics and, therefore, an important and interesting reservoir for the prospection of extracts, fractions,

and biocidal compounds. These materials might be used in the formulation of new eco-pesticides, following the global market trend that arises from society's growing demand for safer food and environmentally friendly practices [90].

The use of plant extracts, as a source of bioactive compounds in the suppression of phytopathogens, represents another possibility to be used in the management of agricultural pests [91]. However, some points deserve attention regarding the use of plant extracts as a basis for the development of natural pesticides. They are (i) the availability of plant material considering the seasonality of the crop in question, (ii) the search for biocidal action standardization showed by plants of the same species, but coming from different regions, (iii) the obstacles in the production of extracts on a large scale, (iv) the low residual characterizing a shorter time of action and, therefore, the demand for a greater number of applications, and (v) the restrictions that come up against the legislation concerning the registration of these products as they fall under the pesticide law.

In short, the implementation of natural pesticides in the production process, replacing synthetic agrochemicals whenever possible, is highly required. The planning to reduce the population of plant parasites must be prioritized and carried out rationally. Furthermore, the biology of the pathogen, the different methods of control, and their respective tactics must still be considered and could contribute to the minimization of the environmental imbalance. The joint implementation of these actions may strengthen the implantation of preventive measures and restrict the use of curative agricultural practices.

Purslane extract can be used as a pesticide as a pest control measure [4, 92]. Studies have shown that the methanolic extract of *Portulaca oleracea* showed high contact toxicity and dichloromethane exhibited antifood toxicity against the *Aphis gossypii* pest that affects cotton [4]. Tayyab et al. [93] carried out a study with 40 species of indigenous plants, including purslane, evaluating the insecticidal potential of acetone extracts. About 41% death rate was observed for the cotton aphid using 10% acetone extract obtained from purslane leaves [93].

6. Conclusions and future perspectives

Purslane is a multipurpose plant species source of many known bio-molecules and features of interest to several distinct industries—pharmaceutical, chemical, food, and feed, just to name a few. It is one of the most used medicinal plants, according to the WHO; but, it is also the eighth most common weed in the world. As an invasive weed, purslane raises several concerns when considered to be grown outdoors, in open fields. Because of that, it has been subject of increasing interest in the context of controlled-environment agriculture, where it delivers very high biomass productivity. Within the scope of controlled-environment, purslane could be produced in greenhouses, in containers (vertical farming systems), or in bioreactors (cell suspensions).

After performing a bibliometric analysis of purslane *in vitro* tissue culture and genetic modification/editing, we came across with very little scientific data available. To exploit the biotechnological potential of purslane, as a source of valuable bio-molecules for the many different industries, it is imperative to change this scenario. Among the key initiatives necessary to do so there are: (a) a Whole Genome Project to generate and make available a reference genome for this plant species; (b) a reliable and efficient protocol for genetic modification/editing, able to produce a large

number (hundreds or thousands) of mutants (genetically modified/edited) per period (a few months or a year); and (c) to build up a multi-omics database harboring information regarding the response of this plant species to many environmental conditions.

Other initiatives, such as the generation of haploid/di-haploid individuals; the production and maintenance of cell suspensions; the production, maintenance, and characterization of purslane's extract library, would be also of great value to exploit the biotechnological potential of this important plant species.

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Conflict of interest

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