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Salinity and medicinal plants: Challenges and strategies for production

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Abstract. Medicinal plants, since antiquity, have been relevant due to their therapeutic properties, are widely used for the prevention and treatment of diseases. However, the growth and production of these plants are impacted by a notorious environmental stressor, salinity. In this sense, this study aimed to review the impacts of salinity on plant development, the deleterious effects of this environmental stressor on the production of medicinal plants, and the production strategies of these species in saline conditions. Areas with salt excess problems have been increasing all over the planet, mainly due to human actions such as inadequate irrigation management. Salinity impacts plants at different times, the first phase results from osmotic stress, while the second derives from the ionic toxicity of the salt constituents. In medicinal plants, saline stress causes damage from germination to changes in morphological characteristics, physiology, nutrient concentration, and productivity. However, in some species, exposure to moderate degrees of salinity was positive for medicinal quality, with an improvement in the constitution of the essential oil. Strategies for the production of medicinal plants in saline conditions are reported in the literature, such as the use of amino acids (such as proline and betaine glycine), salicylic acid, arbuscular mycorrhizal fungi, resistance inducing genes, and projected nanoparticles. These strategies may represent an option for agricultural production in marginal areas and with waters with higher levels of salts.

Keywords: Salt stress, Glycophytes, Tolerance induction.

Contextualization and analysis

Since ancient times, medicinal plants have played a vital role in human societies, used in the prevention and treatment of diseases. Whole plants or their specific parts can be used for medicinal purposes (Wang et al., 2020). Currently, the use of medicines based on medicinal plants is expanding worldwide, used by millions of people who resort to this category of product for the treatment and prevention of various diseases (Romano et al., 2021). Thus, it is evident that these plants present, besides therapeutic benefits, significant economic and social importance (Wang et al., 2020).

In the last decade, there has been a great wave of public acceptance and interest in health treatments based on the use of medicinal plants, the so-called "natural" therapies, which have been gaining widespread use and consolidating in several countries. Due to this and other reasons, medicinal plants are currently the object of great interest in research in different areas (Romano et al., 2021).

The active compounds present in most parts of medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents in different parts of the world. It is noteworthy that these plants are used as medicines in almost all cultures, especially in the Middle East, Latin America, Africa, and Asia, where more than 85% of the population depends almost predominantly on this type of resource, notably herbal medicines, for their health needs (Jamshidi-Kia et al., 2018).

The medicinal properties of these plants derive from the production of secondary metabolites (Dar et al., 2017). These organisms are living chemical factories for the biosynthesis of a wide variety of these types of metabolites, which form the basis of many commercial pharmaceutical drugs as well as herbal remedies derived from medicinal plants (Li et al., 2020). Not surprisingly, most of the important drugs developed in the last 50 years, which have revolutionized modern medical practice, have been isolated or derived from plants (Dar et al., 2017).

The different chemical constituents of medicinal plants possess biological activities with relevant importance for human health, isolated and processed both in the pharmaceutical and food industries, as well as in the perfumery, agrochemical, and cosmetic industries, constituting a very economically significant resource (Li et al., 2020).

In medicinal plants, the biosynthesis of secondary metabolites, such as, for example, essential oils and phenolic compounds and their components, are strongly affected by environmental factors; and of the most impacting environmental constraints for these plants, salinity is one of the most important (Bahcesular et al., 2020). Salt stress affects the growth of medicinal plants, negatively impacting their development and final yield. It also alters the biochemical properties of these plants depending on the type of plant species affected (Miransari et al., 2021). The effects of salt stress on crops, including medicinal plants, are more prominent in arid and semi-arid regions, where 25% of agricultural land is affected by problems with excess salts (Kotagiri & Kolluru, 2017).

Efforts in researching the impacts of salinity on plant development have mostly focused on agricultural, forage, and biomass species for fuel production. However, little work has been done to explore the effects of salinity on medicinal plant cultivation (Muhammad & Hussain, 2010; Aghaei & Komatsu, 2013; Izadi-Darbandi & Mehdikhani, 2018). In this sense, this study aimed to review the impacts of salinity on plant development, the deleterious effects of this environmental stressor on the production of medicinal plants, and the production strategies of these species under saline conditions.

Salinity and Plant Development

About half of the earth's surface presents problems of aridity or semi-aridity. However, the agricultural areas of these regions are those that achieve the best production rates, provided there is sufficient water for irrigation. However, the use of irrigation without proper management and coupled with other environmental factors can lead to another serious problem for agriculture, soil salinization (Fita et al., 2015). Currently, approximately 20% of irrigated areas are affected by problems with salts and annually 1.5 million hectares of arable areas are becoming unsuitable for agricultural production due to high levels of soil salinity (Hossain, 2019). Estimates further indicate that up to 50% of irrigated areas may be affected by problems with excess salts very shortly (Fita et al., 2015).

In this scenario, salinization is recognized as the main threat to environmental resources and human health in many countries, affecting almost 1 billion hectares worldwide, representing about 7% of the continental extent of the planet (Shrivastava & Kumar, 2015). Globally, soil salinity generates annual losses of $12 to $27.3 billion due to reductions in crop productivity (Qadir et al., 2014).

Commonly, saline soil is generally defined as one in which the electrical conductivity of the saturation extract in the root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at a temperature of 25 °C, in addition to having a percentage of exchangeable sodium of 15%. At this electrical conductivity, the yield of most agriculturally important species is already reduced (Shrivastava & Kumar, 2015).

Soil salinity can be classified into primary salinity and secondary salinity (Yan et al., 2015). Primary salinity occurs naturally in regions where the original soil material is rich in soluble salts, these soils may be rich in salts due to the constituents of the parent rock, such as carbonate minerals and/or feldspar (Yan et al., 2015; Daliakopoulos et al., 2016). Also, this type of salinity can be triggered by geochemical processes (Yan et al., 2015), or specific formations that can increase the salt concentration in groundwater and therefore in soil layers (Daliakopoulos et al., 2016). Added with a natural trigger of this process is the deposition of sea salt carried by wind and rain and the inundation of coastal lands by tidal water (Hossain, 2019).

The naturally saline and alkaline soils areas total about 6% of the Earth's land surface. These areas are considered marginal and are not cultivated due to their high salinity, since they could not serve as arable soil for major crops (Fita et al., 2015).

As far as it is concerned, secondary salinity is derived from human actions on the soil (Yan et al., 2015). In climates with low rainfall, high evapotranspiration rates, and soil characteristics that restrict salt leaching, irrigation is an important salinization point. Also, interventions that lead to increased soil water accumulation time or limit drainage can lead to salinization (Daliakopoulos et al., 2016).

Soil salinization can also come from other human actions, such as the result of changes in vegetation cover that alter the water balance of the ecosystem. This type of salinization is reported in Australia, where large areas are becoming over-salinity due to the replacement of deep-rooted, perennial native vegetation with shallow-rooted, shallow-rooted agricultural crops (Rath & Rousk, 2015).

Salinization of agricultural areas is now a global problem (Fita et al., 2015; Shrivastava & Kumar, 2015; Hossain, 2019). It affects 5% of land in Africa, about 20% of arable land in West Asia, 30% of Australian agricultural area, and the 17 western states of the USA (Rath & Rousk, 2015). In Europe, the effects of soil salinization are even more evident, affecting significant parts of Italy, Spain, Hungary, Greece, Cyprus, Portugal, France (west coast), the Dalmatian coast of the Balkans, Slovakia, and Romania. Also, Northern European countries such as Denmark, Poland, Latvia, and Estonia are facing similar problems (Daliakopoulos et al., 2016).

Almost all of the agricultural and forage species used in modern agriculture are glycophytes, meaning that they are salt-sensitive and can withstand only a very limited concentration of salt in their growth media. Among these species are the most widely cultivated in the world for food production, for example, rice, maize, sugarcane, potato, soybean, and beans (Panta et al., 2014).

The excessive presence of salts affects all stages of plant development, including germination, vegetative growth, and reproductive development (Shrivastava & Kumar, 2015). In most cases, this damage is associated with excessive accumulation of Na^+ and Cl^- in metabolically active intracellular compartments (Zhao et al., 2020).

However, this salt sensitivity varies during various stages of plant growth. Usually, plants at early growth stages, such as seedlings, are more sensitive to salt stress than plants at later stages (Zörb et al., 2018). Soil salinity imposes several deleterious effects on the plant, such as ionic toxicity, osmotic stress, nutrient deficiency (N, Ca, K, P, Fe, Zn), and oxidative stress on plants, further limiting soil water uptake (Shrivastava & Kumar, 2015).

Plant responses to soil salinity occur in two phases (Butcher et al., 2016). The first phase, which occurs within minutes or a few days after exposure to salts, results from osmotic stress, caused by sodium ions, which leads to the closure of stomata and inhibition of leaf expansion. In this first phase, the negative influence of salinity on the plant water ratio is also observed (Kotagiri & Kolluru, 2017). The second phase involves the ionic toxicity of salt constituents (Butcher et al., 2016). Accumulation of toxic ions occurs mainly in the older leaves, causing premature senescence, declines in productivity and biomass production, and plant death (Kotagiri & Kolluru, 2017).

Photosynthesis is also severely affected by salinity since it leads to a reduction in leaf area, chlorophyll content, and stomatal conductance of plants (Shrivastava & Kumar, 2015). It is noteworthy that in leaves of plants more sensitive to this environmental stressor, photosynthesis is inhibited (Zörb et al., 2018).

Also, negative effects of salinity are reported on plant reproductive development, causing damage to microsporogenesis and stamen filament elongation, increased programmed cell death in some tissue types, abortion of ovules, and senescence of fertilized embryos (Shrivastava & Kumar, 2015).

However, it should be considered that the degree to which salinity impedes crop productivity is highly variable and dependent on many factors, such as soil texture and water content, soil nutritional status, species and variety, plant growth stage, pest pressures, and the ions that contribute to salinity (Butcher et al., 2016).

In crops, the characteristics associated with yield are inversely related to abiotic stress conditions, such as salt stress during the development of the plant. Thus, if salt stress leads to a reduction in growth at an early stage of plant development, consequently sharp declines in yield are expected, as well as, the plant products of interest will be compromised in quality and quantity (Zörb et al., 2018).

Deleterious Effects of Salinity on Medicinal Plants

In medicinal plants, salinity impacts in different ways, however, the damage is generally reported on morphological characteristics such as leaf number, leaf area and leaf biomass (Yu et al., 2015), physiology (Kotagiri & Kolluru, 2017), nutrient concentration in tissues (Ramezani et al., 2011; Hejazi-Mehrizi et al., 2021) and productivity (Heidari & Sarani, 2012; Rahimi-Dehgolan et al., 2012).

Under field conditions, the first reaction of plants when exposed to high salinity is the reduction of seed germination (Minhas et al., 2020), a reaction also reported for medicinal plants. Hokmalipour (2015) evaluated different salinity levels (0, 2, 4, 6, 8, and 10 dS m^{-1}) on germination and seed vigor of three medicinal plants: chicory (*Cichorium intybus* L.), cumin (*Cuminum cyminum* L.), and fennel (*Foeniculum vulgare)*. The results obtained showed that salinity reduced seedling length, aerial part length, root length, germination percentage, germination rate, seedling dry, and fresh mass. Deleterious results were also observed for the vigor of these seeds, in which a reduction compared to the control treatment was observed of 74, 72, and 47% for fennel, cumin, and chicory, respectively. Seeds of fenugreek (*Trigonella foenum-graecum* L.), dragonhead (*Dracocephalum moldavica* L.), savory (*Satureja hortensis* L.), and dill (*Anethum graveolens* L.), medicinal species common in the Middle East, were treated with various concentrations of NaCl (0, 40, 80, 120 and 160 mM L^{-1}), and in all species, an increase in NaCl concentration led to a decrease in germination and seedling growth (Saberali & Moradi, 2019).

For *Aloe vera* grown at different salinity levels (3, 6, 9, 12, 15, 18, and 21 dS m^{-1}), salinity was observed to negatively influence plant growth and morphological characteristics, and biomass (Rahimi-Dehgolan et al., 2012). However, studies such as those by Murillo-Amador et al. (2014) show that *A. vera* plants under moderate salt stress can respond favorably to certain dosages of NaCl.

In chamomile (*Matricaria chamomilla* L.), Heidari and Sarani (2012) showed that increasing salinity from 0 to 150 mM NaCl, leads to a decrease in aerial part fresh mass (76.3%) and an increase in root fresh mass (53.8%).

In three plant species of the genus *Plectranthus* (*P. amboinicus, P. barbatus and P. grandis*), popularly known as boldo, Freitas et al. (2014) observed that salt stress reduced plant growth and influenced dry matter partitioning, the roots being more affected than the aboveground part. An increase in electrical conductivity also led to a reduction in K content^{+} in leaves. Similar results were observed in rosemary (*Rosmarinus officinalis* L.), in which reduced dry matter was also observed in plants under higher saline concentrations and reduced K concentrations in the plant area (Hejazi-Mehrizi et al., 2021).

In mint (*Mentha canadensis* L.), one of the most important plants for essential oil production, NaCl concentrations higher than 50 mM were already sufficient to lead to a reduction in biomass and water content of all plant parts. Salinity also reduced plant height, root and branch length, number of branches, nodes, and essential oil content (Yu et al., 2015).

Kotagiri and Kolluru (2017) investigated the effect of different salinity concentrations (100, 200, and 300 mM NaCl) on carbohydrate content, plant growth, leaf area, and biomass of five species of the *genus Coleus* (*C. aromaticus*, C. *amboinicus, C. forskohlii,* C. *zeylanicus,* and *C. barbatus*, and found that salt stress significantly reduced plant growth, leaf water potential, and relative water content in all species.

In *Thymus vulgaris* and *Thymus daenensis*, two of the most important medicinal and aromatic plants of Iran, Bistgani et al. (2019) evaluated the effects of saline irrigation using different concentrations of NaCl (0, 30, 60, and 90 mM) on the growth and physiological characteristics of these species. The results indicated that 60 and 90 mM NaCl concentrations significantly decreased plant dry matter production by about 28 and 40% in *T. vulgaris* and 34 and 39% in *T. daenensis*, respectively, compared to untreated plants. NaCl application also induced an increase in Na content^{+} in the aerial part and leaves, while K content^{+} and Ca content^{2+} decreased with salt stress.

Roodbari et al. (2013) evaluated salinity levels of 0, 50, 100, and 200 mmol NaCl on growth parameters and essential oil percentage of *Mentha piperita* L., one of the most important essential oil-producing plants of Iran. The results showed that salt stress significantly affected stem length, root length, aerial wet weight, root wet weight and aerial dry weight, root dry weight, internode length, biomass, and percentage of essential oil in this species.

When evaluating the effect of salinity on some physiological and morphological characteristics of the medicinal plant *Echium amoenum*, Ramezani et al. (2011) observed that the application of saline water significantly reduces all the morphological characteristics understudy, besides, lower concentration of K^{+}, Ca^{2+} and Mg^{2+} was observed in the root and stem. Also, as salinity increased, the concentration of proline and total soluble sugars in the leaves increased significantly compared to the control, demonstrating that the accumulation of proline and soluble sugars are good indicators of salinity tolerance (Ramezani et al., 2011).

It is also important to highlight the contrasting effects of the use of saline water on some medicinal plant species. For example, Yan et al. (2016) through hydroponic and field experiments, analyzed the effects of salt stress on the accumulation of chlorogenic acids in honeysuckle (*Lonicera japonica* Thunb.) and observed that although salinity led to a reduction in the biomass of this species, salt stress improved its medicinal quality by promoting the accumulation of these acids. Similar results were observed for the essential oil constitution of basil (*Ocimum basilicum* L.) subjected to moderate salinity conditions (Hassanpouraghdam et al., 2011). In such cases, the stimulus to the improved performance of essential oil production under moderate degrees of salinity may be derived from a higher density of oil-producing glands, as well as, to an increase in the absolute number of the development of these glands before leaf emergence. Also, the increase in oil content in some of these plants subjected to salt stress can be attributed to a reduction in the concentration of primary metabolites, thus making the intermediate products available for the synthesis of secondary metabolites (Ahl & Omer, 2011).

Strategies for the Production of Medicinal Plants in Saline Conditions

The management of saline soil recovery is based on the adjustment or installation of drainage systems that allow the washing of salts present in the soil, adding to this the need for better management of irrigation water, which leads to obtaining salinity levels that are acceptable for crops. However, growing crops under saline conditions is a more pragmatic approach, in which ways of adapting crops to these conditions are sought. In this sense, the cultivation of halophytic plants, species that are more tolerant to excess salts in the soil, as well as the cultivation of perennial plants with deep roots, stand out (Hossain, 2019).

It is noteworthy that halophyte plants can be cultivated not only for food and fuel production purposes but also for medicinal purposes. In traditional medicine, some species play an important role in the treatment of diseases in different parts of the world, for example, *Ipomoea pes-caprae*, *Mesembryanthemum crystallinum,* and *Tamarix gallica* (Panta et al., 2014).

In plants, a crucial physiological trait for tolerance to salinity is the accumulation of organic compounds, such as certain amino acids (proline, proline betaine, glycine betaine, and β- alanine betaine) and soluble sugars (fructose, glucose, fructans, raffinose, and trehalose). The presence of these compounds, mainly in stress conditions for the plant, allows the maintenance of the turgescence potential, diminishing the osmotic potential and minimizing the deleterious effects of Na ions$^+$ against ribosomes and proteins. Recently, the exogenous application of proline and betaine glycine has also been considered as a strategy to improve plant production in salinity soils (Kamran et al., 2020).

In safflower (*Carthamus tinctorius* L.) seedlings submitted to salinities of 0, 50, 100, and 150 mM NaCl, the exogenous application of glycine betaine led to an increase in the activity of catalase (CAT) and superoxide dismutase (SOD) enzyme, while reduced the activity of peroxidase (POD) in plants under salt stress, thus demonstrating an increase in salt tolerance in this species with the use of this amino acid (Alasvandyari et al., 2017). The exogenous application of proline had positive effects in *Aloe vera* plants subjected to salinity, mitigating the negative effects of this stressor by improving the K/Na ratio, accumulation of carotenoids and phenolics, and the activity of elimination of ROS, which resulted in better functioning of PSII (Nakhaie et al., 2020).

Salicylic acid (AS) is an antioxidant phytohormone naturally produced by plants, presents recognized participation in the local and systemic defense response against pathogens, and lately has been used to reduce the harmful effects of salt stress (Batista et al., 2019). The application of this phytohormone can alleviate salt stress by modifying the chemical composition, gene expression, and bioactivity of plant secondary metabolites (El-Esawi et al., 2017). In the medicinal and aromatic plant *Egletes viscosa* (L.) Less, exogenous application of AS was able to increase K^+ levels and decrease the overproduction of H_2O_2 in plants subjected to salt stress, increasing key metabolites, which led to a higher net photosynthetic rate (Batista et al., 2019).

In rosemary (*Rosmarinus officinalis* L.), treatments with 200-300 ppm of AS allowed reduction of salinity effects in this species, with significant increases in the contents of phenolics, chlorophyll, carbohydrates, and total leaf proline compositions, being also observed reduction of Cl^- and Na^+ in treated plants. AS also led to the activation of antioxidant mechanism pathways, thus stimulating the activity of antioxidant enzymes (El-Esawi et al., 2017).

In *Tanacetum parthenium*, a valuable medicinal plant of the Asteraceae family with various pharmaceutical and therapeutic properties, the use of AS via foliar application on salinity-grown plants improved plant height, number of leaves, and shoots, and fresh and dry weight. Moreover, improvements were observed in the essential oil content of this species (Mallahi et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are considered bioremediators of soil salinity in plants (Wang et al., 2018). These fungi use several mechanisms to mitigate the effects of salt stress, such as increasing nutrient and water uptake, maintaining osmotic balance, stimulating antioxidant activities to protect the plant against reactive oxygen species (ROS) damage, increasing photosynthetic rate, and regulating hormone levels (Dastogeer et al., 2020).

The use of two AMFs, *Funneliformis mosseae,* and *Diversispora versiformis*, alone and in combination, were shown to be efficient in the growth and nutrient uptake of the medicinal plant *Chrysanthemum morifolium* grown for five months under different salinity levels (0, 50, and 200 mM NaCl). Root length, shoot number, root dry matter, total dry matter, and N concentration were higher in mycorrhized plants than in non-mycorrhized plants under moderate salinity conditions (Wang et al., 2018).

Promising results of AMF use are also reported for *Dracocephalum moldavica* L., in which inoculation with *Funneliformis mosseae* and *Claroideoglomus etunicatum* increased the growth parameters and the salinity tolerance of this medicinal plant in all saline levels tested. It was also observed that these AMFs triggered non-enzymatic antioxidant activity by accumulating flavonoids in the roots and shoots and phenol in the roots, improving growth parameters and reducing root cell death in comparison with non-mycorrhized plants (Alizadeh et al., 2021).

In basil (*Ocimum basilicum* L.), mycorrhizal inoculation significantly increased chlorophyll content and water use efficiency under salt stress. Basil plants appear to have a high dependence on AMF, which improved plant growth, photosynthetic efficiency, and gas exchange under salt stress (Elhindi et al., 2017).

In recent years, there has been a considerable increase in the number of works that address salinity tolerance from a molecular point of view, to search for genes that may be useful in the search for resistant genotypes. The identification of these genes would provide valuable information about the molecular and genetic mechanisms involved in the salt tolerance response, as well as, also provide important resources for plant breeding programs (Hernández, 2019).

Many salt-responsive proteins have already been identified in crops, such as heat shock proteins, pathogen-related proteins, protein kinases, ascorbate peroxidase, osmotin, ornithine decarboxylase, and some transcription factors, which seem to give these crops the ability to resist salt stress (Aghaei & Komatsu, 2013).

In *Salvia miltiorrhiza* Bunge, a plant highly valued for its roots in traditional Chinese medicine, the *SmLEA* gene originating from abundant late embryogenesis (LEA) proteins was shown to have properties to attenuate the effects of salinity in this plant (Wu et al., 2014). For this and similar cases, the challenge for science now is to use these proteins and their corresponding genes, to transform salt-sensitive crops into tolerant crops shortly (Aghaei & Komatsu, 2013).

In recent years, to enhance strategies for agricultural production under saline conditions, engineered nanoparticles (NPs) have been widely employed and considered as effective strategies in this regard. Titanium dioxide and selenium nanoparticles were tested as mitigators of adverse effects of salinity in stevia (*Stevia rebaudiana* Bertoni) and led to increased growth, photosynthetic performance, antioxidant enzyme activity, and decreased content H_2O_2. In addition, the increased essential oil content was observed with the use of these materials (Sheikhalipour et al., 2021).

Final Considerations

The increase in areas with salinity problems is currently a global concern due to its potential impacts on the food security of the human population, as well as the associated environmental impacts. Like other crops, medicinal plants are mostly negatively impacted under saline stress conditions, with reflections on reduced growth and reduced biomass production and productivity. However, strategies for production under these conditions have been developed and may represent an option for agricultural production in marginal areas and with waters with higher levels of salts.

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