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Citrus Salinity Tolerance: A Systematic Review of Cultivar Selection Trials, Grafted versus Nongrafted Trees, and Scion Contributions

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***Abstract.* Salinity poses a significant challenge in horticulture, particularly for citrus, one of the most widely cultivated fruit crops. Soil bulk electrical conductivity levels above 1.4 dS/m can impair tree performance, reducing productivity and fruit quality. With increasing occurrences of extreme weather events and competition for fresh water, the identification of salinity-tolerant citrus cultivars is essential for sustainable production. This review examines citrus salinity tolerance trials, emphasizing cultivar selection, the use of grafted vs. nongrafted trees in experimental designs, and the role of the scion in salinity adaptation. Although most studies focus on rootstock influence, emerging evidence suggests that scions also regulate ion accumulation and stress responses. Research on salinity tolerance in citrus has expanded in recent years, with Spain, the United States, and Brazil leading investigations. Although arid and semiarid regions dominate salinity studies, in humid areas such as Florida there is also research interest due to the proximity of citrus production fields to coastal areas. The most frequently measured parameters in salinity trials include leaf and root ion concentrations, gas exchange, dry weight, and growth. A comprehensive assessment of salinity tolerance should integrate measurements that capture both osmotic stress and ion toxicity, including gas exchange, ion accumulation, dry weight, growth, chlorophyll fluorescence, and water/osmotic potential. Further research is necessary to optimize cultivar selection by evaluating both rootstock and scion contributions to salinity tolerance.**

Soil salinity is a widespread global challenge, particularly in irrigated agricultural regions (Stavi et al. 2021). Increasing soil salinity is leading to the loss of an estimated 0.3 to 1.5 million hectares of farmland each year, while also reducing the productivity of an additional 20 to 46 million hectares (Boretti and Rosa 2019). The rise in temperatures, expected extreme precipitation patterns, and rising sea levels caused by climate change contribute to land salinization. Increased evapotranspiration leads to greater salt accumulation in the soil, variable rainfall affects water supply and salt percolation, and saltwater intrusion into freshwater aquifers raises salinity concentrations, thereby degrading water quality for irrigation (Eswar et al. 2021; Hassani et al. 2021). Over time, salts build up in the root zone, negatively affecting tree growth and productivity (Parida and Das 2005). Regions most affected by salinity include arid and semiarid areas, such as the Middle East, South Asia, parts of Australia, the southwestern United States, and the Mediterranean Basin, where evapotranspiration exceeds rainfall. In these regions, agriculture relies heavily on irrigation due to the limited rainfall, which exacerbates salinity issues by introducing additional salts into the soil and restricting their leaching (Naorem et al. 2023). Under certain conditions, salinity issues can also occur in humid regions, particularly when there is poor soil drainage, and shallow water tables. Climate change and increasing competition for water resources are expected to intensify salinity problems in the future (Eswar et al. 2021).

Citrus is one of the most important crops worldwide, ranking third among the most produced fruits (FAOSTAT 2022). Key citrus species include oranges (*Citrus sinensis*), mandarins/tangerines (*Citrus reticulata*), lemons/limes (*Citrus limon* and *Citrus aurantiifolia*), and grapefruit (*Citrus paradisi*), with a global production reaching 47.4 million, 30 million, 10.1 million, and 6.9 million tons, respectively, in 2024 (USDA 2024). Citrus consumption is widely associated with health benefits, particularly due to its high content of vitamin C and other essential nutrients (Richa et al. 2023). These fruits are consumed fresh or as processed products such as juices, jams, and essential oils, and they also have widespread industrial uses in flavorings, cosmetics, and cleaning agents due to their valuable natural oils and extracts (Palazzolo et al. 2013).

Despite its global importance, citrus production faces significant challenges, with salinity being one of the most critical abiotic stresses (Donkersley et al. 2018). Citrus trees are particularly sensitive to salinity, levels of salinity of the soil solution above 1.4 dS/m can negatively affect tree performance (Maas 1993). The accumulation of salts in the soil disrupts the osmotic balance, hindering water and nutrient uptake, and can lead to ion toxicity when excessive amounts of sodium (Na^+) and chloride (Cl^-) accumulate in plant tissues, ultimately decreasing growth and fruit yield (Ziogas et al. 2021). Management practices to mitigate salinity include the selection of tolerant cultivars, adjustment of irrigation volumes, application of soil amendments, and use of alternative water sources for irrigation (Boman et al. 2005). This review focuses on the research related to the selection of salinity-tolerant cultivars in citrus. Understanding which cultivars are better suited to tolerate salinity stress is essential for sustaining citrus production in regions affected by soil salinization (Gupta et al. 2019).

Although citrus is generally sensitive to salinity, some varieties have shown a higher capacity to withstand salt stress. This lower sensitivity can be due to enhanced osmotic adjustment or an adaptative reduction in the uptake of toxic ions (Moya et al. 2002).

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Cultivar trials conducted to identify tolerant cultivars often are carried out under controlled greenhouse conditions by exposing the trees to varying stress salinity levels for quick assessment (Etehadpour et al. 2020; Levy et al. 1999; Vaniés et al. 2018). The evaluation correlates these levels with multiple physiological and tree growth parameters (Ashraf 2004).

Research trials use either grafted or nongrafted trees for salinity tolerance assessments. Most research focuses on the rootstock, which plays an important role in the tolerance of the grafted tree; however, the contribution of the scion is often overlooked (Zrig et al. 2023). The emphasis on the rootstock may lead to an incomplete understanding of the response of the scion/rootstock combination to salinity. Commercial citrus crops are typically grown as grafted trees, a practice that combines parts from two different trees to form a single organism with enhanced traits. This involves the union of a scion, which forms the upper part of the tree responsible for producing fruits and flowers, and a rootstock, which provides the root system and influences factors like water and nutrient uptake, as well as tolerance to environmental stresses generally (Mudge et al. 2009). Nongrafted trees are grown on their own root system, without being combined with a different cultivar. Understanding whether grafted trees should be prioritized in these trials is important to carry out more efficient research.

Since salinity problems have become a growing concern, it is important to assess the current status of citrus cultivar selection trials for salinity tolerance. This involves determining the most effective combination of parameters to identify differences between cultivars, assessing the optimal plant material (grafted or nongrafted) for such studies, and understanding the role of the scion in the contribution to the overall salinity tolerance of the combination scion/rootstock. By addressing these, researchers can improve the accuracy and applicability of salinity tolerance studies and provide better guidance for commercial citrus production in regions prone to salinity stress.

The objectives of this review are 1) to systematically review and summarize the current state of research on salinity stress in citrus focusing on main citrus research areas and on key measurements for tolerance, 2) to evaluate the relevance of using grafted vs. nongrafted citrus trees in salinity studies, and 3) to study the specific role of the scion in salinity stress responses of citrus trees by reviewing the available research focusing on the scion.

Methods

A systematic literature was conducted to assess the current state of research on salinity and citrus. The steps followed in the systematic review are documented as follows.

Three questions were addressed in this study:

Question 1 (Q1): What is the current state of salinity research on citrus?

Question 2 (Q2): Is there an influence of grafting on salt tolerance evaluation?

Question 3 (Q3): What is the role of the scion cultivar on the salinity tolerance of the combination of scion and rootstock?

To address Q1, a series of sub-questions was established to uniformly extract relevant information from the research papers: year of publication, countries leading in research, main measurements used to assess tolerance, and the primary cultivars used (both rootstocks and scions). For Q2, the focus was on determining how many studies used grafted trees in their experiments and how many scions were compared for experimental purposes. Based on the information obtained from Q2, Q3 was formulated. To answer Q3, only papers that assessed more than one grafted scion in the experiments were considered.

Search process

The search process was conducted manually using two primary academic research tools: Google Scholar and Web of Science. Google Scholar was included for its broad access to diverse sources, including emerging studies that may not be captured in traditional databases. Web of Science was selected for its focus on high-quality journals, allowing for the identification of influential studies. The keyword used for both searches was "citrus salinity."

Inclusion and exclusion criteria

Different phases of the search involved including and/or excluding papers, following the PRISMA 2020 methodology (Page et al. 2021) (Fig. 1). Initially, only papers published between 1970 and 2023 and written in English were included in the review. The search process was carried out manually.

Identification

The initial search was conducted by using the keyword and the results were documented as identified papers. Following this, several reports were automatically excluded based on predefined categories: review articles, conference proceedings, meeting abstracts, notes, and retracted publications. This process resulted in 1336 reports being selected for the screening phase.

Screening

The screening process consisted of multiple phases. The first phase focused only on the title; reports that did not match the topic of interest were removed. These included studies on crops other than citrus and reports related to fields other than horticultural science (e.g., genetics, entomology, engineering). During the second phase, duplicated reports from both datasets were removed, and the remaining papers underwent another screening, this time including the abstract. Given that this systematic review focused on the single effect of salinity stress on citrus cultivars, papers that included other factors interacting with salinity—such as drought, flooding, deficit irrigation, application of elements (e.g., nitrogen, calcium, phosphorus), mycorrhizae, biotic stresses, foliar fertilizer—were excluded. In addition, reports on in vitro experiments were removed, as the aim was to focus on greenhouse and field studies. In the final screening phase, the full-text versions of the reports were evaluated. Elimination criteria included incomplete reports and studies with fewer than three replicates. Inclusion criteria were open-access reports and literature reviews. The remaining reports were included in this study.

Inclusion

The inclusion phase was divided into two parts, depending on the research questions being addressed. All reports resulting from the screening, 135 reports, were included to answer Q1 and Q2, as these required a broad overview of knowledge in citrus salinity. To answer Q3, an additional round of elimination was conducted, excluding reports that evaluated only nongrafted trees or included only one grafted scion. Only the documents assessing two or more grafted scions were included, 13 reports.

Data collection

Specific data relevant to the research questions were extracted from each selected paper. This process involved identifying and defining variables to answer the questions. It was essential that the selected papers contained these variables to provide a comprehensive understanding. Qualitative data were categorized and converted into measurable formats where possible (e.g., coding themes or assigning scores). This standardization facilitated uniform data entry, enabling comparison of findings across studies in both qualitative and quantitative analyses.

Q1 data

Trend of published papers over time: The publication year was recorded for each document, and a histogram was used to display the number of publications per year.

Papers published by location. The country where the experiment took place was recorded for each publication.

Measurements to screen salinity tolerance in citrus. Measurements from the Materials and Methods section of each paper were recorded. A list of all measurements carried out in the papers was compiled, and a binary classification was used. If a paper used a specific measurement, a "1" was assigned; otherwise, a "0" was noted.

Q2 data

Grafted vs. nongrafted trees. A binary classification was used, "1" was assigned for studies that used grafted trees, and "0" for nongrafted trees. The percentage of each category was then calculated, reflecting the proportion of studies that used grafted vs. nongrafted trees.

Number of scions. Within the grafted trees category, the number of scions used in each experiment was quantified.

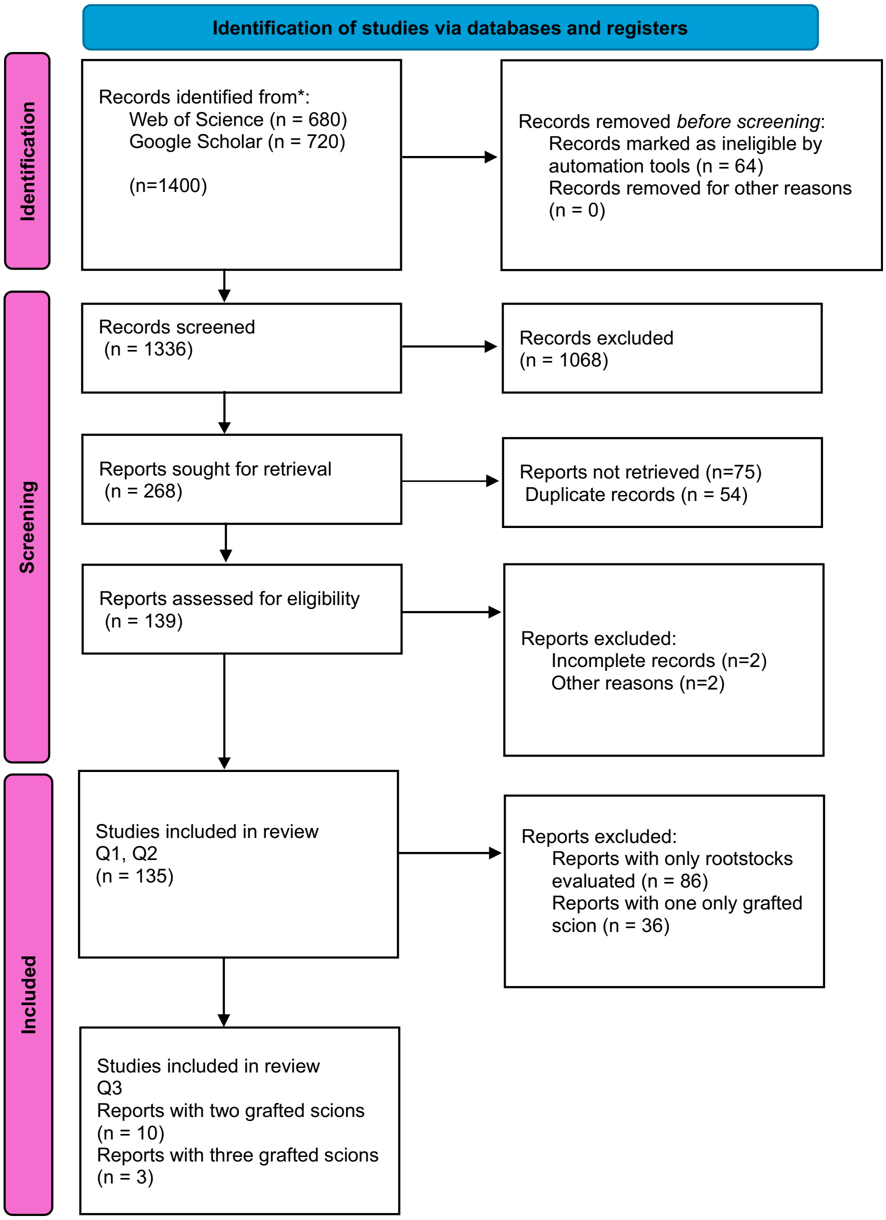

Fig. 1. PRISMA flow diagram for systematic literature review. The diagram illustrates the stages of study identification, screening, and inclusion for a systematic review. It details the number of studies identified via databases and registers, the screening process, and the final number of studies included for analysis. Exclusion criteria for each stage is explained in the Methods section.

Q3 data

To answer this question, the following data were recorded: Author, Year, Number of Scions, Scion Cultivar, Rootstock Cultivar, Salinity Concentrations, and Main Results. The main results focused on variables that were affected by the scion and where a Salinity × Scion interaction was observed in the analysis of variance. These results were recorded categorically.

Results

Published papers over time

The number of publications related to citrus salinity research for 50+ years from 1970 to 2023 reveal distinct trends in research activity over the years (Fig. 2). From the late 1970s through the 1980s, there were relatively few publications, with a total of eight. A gradual increase is observed in the 1990s, reaching 21 publications, followed by another rise in the early 2000s, with 25 publications. This period marks the beginning of a more consistent research effort, with the number of publications fluctuating, but generally increasing over time. Significant peaks in research output are observed in 2015 and 2020, with the highest number of publications being in 2015, up to 14 publications.

Published papers by country

The major contributor to citrus salinity research was Spain, leading the ranking with 39 papers published. This is followed by the United States (16), Brazil (14), Australia (11), and Pakistan (10) (Fig. 3). The rest of the countries have fewer than 10 publications: France and India (6); Turkey and Israel (5); Iran (4); Greece (3); Malaysia (2); and Bangladesh, Japan, Jordan, Morocco, and the United Arab Emirates (1 each). In contrast, large areas of the world, particularly Africa and Central Asia, appear in gray, indicating no research output from these regions.

Measurements to screen salinity tolerance in citrus

A network plot was used to visualize the main variables used in citrus salinity research (Fig. 4). The network shows the frequency of how many times a variable was measured and displays the relationship between different variables illustrating how often they co-occur in the literature. Each node represents a variable, and the size is proportional to the frequency with which that variable appears in the literature. Larger and darker nodes indicate more frequently measured variables. On the other side, the lines connecting the nodes represent the co-occurrence of these variables within the same studies. Thicker and darker lines indicate higher co-occurrence weight, meaning those variables are often used together. We identified several core variables such as leaf ion concentrations, gas exchange, growth, plant dry weight, and root ion concentrations (listed from higher frequency to lower) that are situated at the center of the network. In addition, these variables have a high co-occurrence weight, showing that they are often used together in literature. Leaf ion concentrations and gas exchange are the most correlated parameters, followed by leaf ion and growth, and leaf ion and plant dry weight. The periphery of the network shows the measurements that are less frequently used and have fewer connection to other variables.

Published papers using grafted vs. nongrafted trees and number of scion cultivars

There is a larger proportion of the studies in citrus salinity research that used nongrafted trees (60.3%) compared with grafted trees (39.7%) (Fig. 5). Most studies that used grafted trees used a single grafted scion, as shown in Fig. 6. In contrast, a smaller number of studies explored the use of two grafted scions with nine studies, and only four studies involved three grafted scions. Then, these studies were classified into three categories: studies that used reciprocal grafts, greenhouse studies, and field studies. Reciprocal grafts are grafting combinations in which two genotypes are used in both positions, each serving once as the scion and once as the rootstock.

Effect of grafted trees vs. nongrafted trees in the tolerance of salinity stress

It has been shown that nongrafted trees and grafted trees respond differently to salinity stress and that the consequences of those

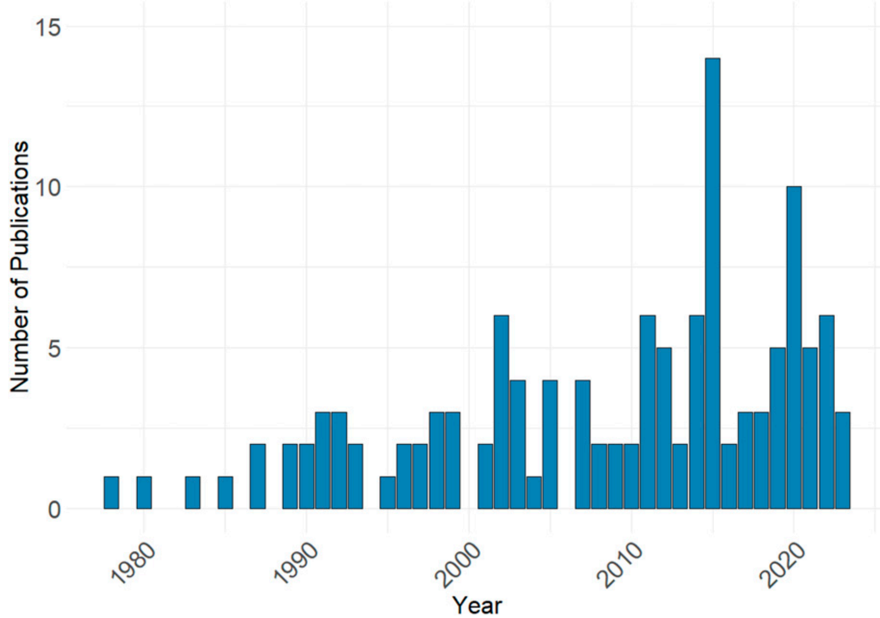

Fig. 2. Temporal distribution of scientific publications on citrus salinity research from 1970 to 2023.

responses are affected by the cultivar combination of rootstock and scion. Moya et al. (2002) found that grafted trees of reciprocal grafts between ‘Carrizo’ and ‘Cleopatra’ were less tolerant than their respective nongrafted trees and that higher water usage and higher leaf chloride were the cause of the lower tolerance. On the other hand, Bleda et al. (2011) observed that in the same comparison between reciprocal grafts and nongrafted trees, grafted trees showed more tolerance than nongrafted trees. In this case, the cultivars used were ‘Cleopatra’ and ‘Alemow’ (Table 1).

Effect of the scion on the tolerance to salinity of the scion-rootstock combination under greenhouse trials

Most studies primarily focus on the effects of salinity on various aspects of citrus scion-rootstock combinations. The titles emphasize mostly the salinity tolerance. The studies range from 1985 to 2023 with a noticeable gap between 1998 and 2020. Common scion cultivars mentioned include lemon (e.g., ‘Fino’, ‘Eureka’), sweet orange (e.g., ‘Valencia’), and mandarin cultivars. There is a focus on lemon cultivars in four out of the seven reports. ‘Sour orange’ appears frequently as the rootstock chosen for the experiments. Other common rootstocks include ‘Cleopatra’, ‘Rough lemon’, and ‘Carrizo citrange’. Salinity levels used in the treatments typically include a range from 0 mM (control) to ∼80 to 90 mM, with commonly studied concentrations being 40 mM and 50 mM. Ion content (Cl^-, Na^+) is the most measured parameter, indicating a strong interest in understanding how salinity affects ionic balance in scion/rootstock combinations. Gas exchange parameters [net photosynthesis (A), stomatal conductance (g_s), and transpiration (E)] and chlorophyll content (Chl) are also frequently measured, followed by water potential (Ψ) and growth metrics to determine the physiological impacts of salinity. Papers that assessed accumulation of Na and Cl in leaves reported that Na, Cl, or both were found to be different among the scions evaluated. Studies that assessed chlorophyll contents show that the scion had an effect on this parameter and that it was often related to the concentration of ions found in the leaves, the higher accumulation of ions in plant tissue the higher decrease in chlorophyll content. Chlorophyll was often also affected by the rootstock. Regarding growth and water relations, although some studies showed an interaction between scion and salinity, this result was not consistent among all the studies (Table 2).

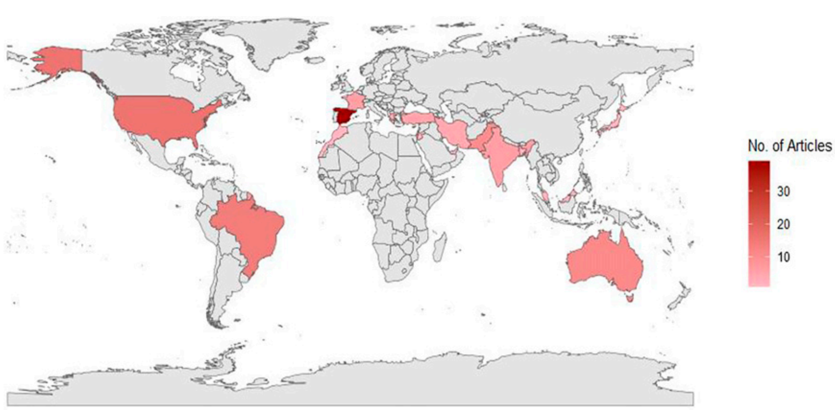

Fig. 3. World map illustrating the distribution of scientific publications on citrus salinity by country after the PRISMA screening process. Countries shaded in darker colors indicate higher publication activity, reflecting a greater number of relevant studies. Number of papers per country: Spain (39); United States (16); Brazil (14); Australia (11); Pakistan (10); France and India (6); Turkey and Israel (5); Iran (4); Greece (3); Malaysia (2); and Bangladesh, Japan, Jordan, Morocco, and the United Arab Emirates (1 each).

Discussion

Q1: What is the current state of salinity research in citrus?

Overall, the data indicate a growing interest in citrus salinity research, with more publications in recent years compared with earlier decades. Although this trend suggests that the topic has gained increasing attention in the scientific community, it also could be partly attributed to the overall increase in scientific publications across all fields in the past decades. As salinity issues become more prevalent, research is increasingly focused on identifying salt-tolerant citrus cultivars and understanding their tolerance mechanisms through cultivar trials.

The global distribution of research in citrus salinity is highly heterogeneous, with certain regions exhibiting intense research activity and others showing minimal engagement. A significant factor influencing the focus on salinity research in each area is the region’s climate. Spain, which has the highest number of publications, features an arid climate in the southeastern region, where most citrus production occurs (FAO 2021). Arid and semiarid regions are particularly prone to salinity issues due to higher rates of evapotranspiration compared with precipitation, leading to salt accumulation in the soil along with challenges related to water scarcity (Perri et al. 2022). Interestingly, the United States and Brazil also rank among the top three countries in salinity research, despite most of these areas having nonarid climates. In the United States, most research has been conducted in Florida and Texas. Florida is characterized by a tropical climate in the south and temperate in the rest, whereas most of Texas is defined as temperate climate (Beck et al. 2018). The problem emerges from the low quality of the water resources available due to saltwater intrusion, and in some cases the weather conditions of specific areas (Jasechko et al. 2020). Florida experiences a dry season that lasts from October to May, which contributes to salinity buildup as a result of irrigation with high-salinity water (Abiy et al. 2019). In the case of Brazil, although most of the country falls under tropical and temperate climates, there is a small region, in the eastern part of the country that is classified as arid (Beck et al. 2018). That is where the state of Paraíba is located. Remarkably, 12 of the 14

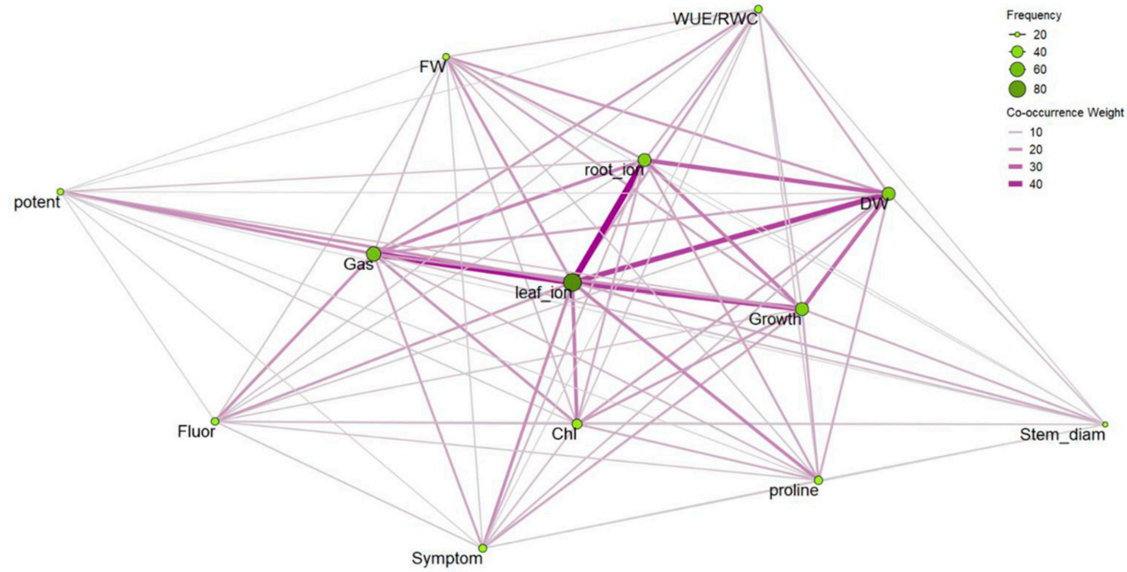

Fig. 4. Network graph of the main measurements used in citrus salinity research. Each node represents a specific measurement, with the size and color of the node corresponding to its frequency of occurrence in the literature. The edges between nodes indicate the co-occurrence of measurements within the same studies, with thicker and darker edges representing higher co-occurrence weights. The co-occurrence represents the frequency in which the two connected parameters are measured together in the literature. DW = dry weight; WUE/RWC = water use efficiency/relative water content; FW = fresh weight; Gas = gas exchange (e.g., photosynthetic rate, stomatal conductance); Chl = chlorophyll content; Fluor = chlorophyll fluorescence (F_v/F_m); Symptom = visual symptoms of salinity stress; Leaf_ion = ion concentration in leaves (e.g., Na^+, Cl^-); Root_ion = ion concentration in roots; Proline = proline content; Stem_diam = stem diameter; Potent = leaf/stem water, pressure and/or osmotic potential; Growth = overall plant growth parameters (e.g., height, canopy size).

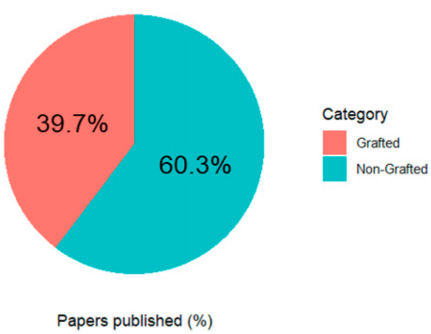

Fig. 5. Percentage of studies included in this systematic review after the PRISMA screening process, categorized based on the use of grafted vs. nongrafted citrus plants in salinity research.

salinity-related publications from Brazil had this state as the experimental site, highlighting the salinity issues prevalent in this region. Australia presents a unique case, where citrus

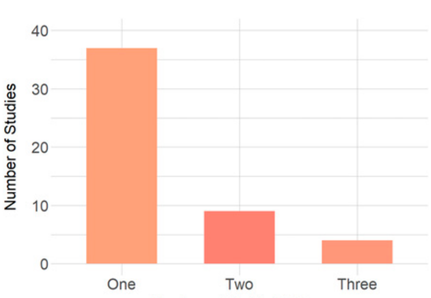

Fig. 6. Number of studies based on the number of scion cultivars used in salinity tolerance trial, after excluding studies with nongrafted trees using the PRISMA screening process.

salinity research does not follow a clear climate pattern, as research is spread across the country, encompassing diverse climatic conditions ranging from tropical to arid. However, most of the research is located in Murray-Darling Basin regions. The Murray-Darling Basin is naturally saline due to primary salinization, which results from salt stored in groundwater systems. In addition, human-induced salinization, primarily driven by irrigation with low water quality and fertigation, has significantly worsened the issue, increasing the salinity of the basin's water. At the same time, the basin remains one of the main sources of irrigation. This creates a self-inflicted challenge, as water quality for irrigation continues to salt built-up in the soil, further threatening crop productivity—particularly for salt-sensitive crops like citrus. In contrast, Pakistan, which also exhibits significant research activity, is classified as an arid desert, with very low precipitation throughout the year. India has a wide spectrum of climates, ranging from arid to tropical, with most areas experiencing salinity issues falling within the arid and semiarid climate zones. Other countries contributing to citrus salinity research include temperate nations like France and Greece, as well as other arid regions like Iran and Israel (Beck et al. 2018).

Among the countries assessed in this review, Brazil, the United States, and Spain are the top citrus producers (FAO 2021). The high level of research activity in the impact of salinity in citrus production aligns with the economic importance of citrus in these countries. As a result, it makes sense that they allocate more funding to studying cultivar salinity tolerance to improve citrus fruit production under salinity stress. On the other hand, Australia, one of the countries with lower citrus production, shows peak research activity between 1980 and 2000, reflecting historical concerns rather than current production priorities. India, the third-largest citrus producer, exhibits medium research activity in salinity, likely due to other pressing agricultural challenges such as soil quality, pests and diseases, and water availability (Singh 1998). Interestingly, no salinity research was found from China, despite it being the leading global citrus producer, which may suggest other research priorities in citrus production or that most of the research in salinity is carried out in the native language and was out of the scope of this review. The focus on selecting salinity-tolerant cultivars is essential for sustaining citrus production in these regions.

To effectively assess cultivar salinity tolerance, researchers rely on measurements that provide insights into the physiological and biochemical responses of citrus trees under salt stress. In this review, the most used measurements were identified and quantified. These measurements are crucial for understanding the two primary phases of salinity stress: osmotic imbalance and ion toxicity. The first phase, osmotic imbalance, occurs when high salt concentrations in the soil increase, affecting the capacity of the tree for water uptake (Balasubramaniam et al. 2023). The second phase, ion toxicity, arises from the excessive accumulation of salts—primarily sodium (Na^+) and chloride (Cl^-)—in plant tissues, which can cause metabolic disruptions (Parihar et al. 2015). Both phases, individually or together, significantly influence plant behavior and response to salinity stress. Among the

Table 1. Summary of studies on the effects of grafted and nongrafted trees in the response to salinity stress.

Title	Authors	Year	#Scions	Scion cultivar	Rootstock cultivar	Treatments (mM NaCl)	Parameters	Results
Transmissible salt tolerance traits identified through reciprocal grafts between sensitive Carrizo and tolerant Cleopatra citrus genotypes	J.L. Moya, F.R. Tadeo, A. Gómez-Cadenas, E. Primo-Millo, and M. Talón	2002	2	‘Cleopatra’ mandarin (*Citrus reshni*) and ‘Carrizo’ citrange (*Citrus sinensis* × *Poncirus trifoliata*)	‘Cleopatra’ mandarin (*C. reshni*) and ‘Carrizo’ citrange (*C. sinensis* × *P. trifoliata*)	0 mM, 60 mM	Cl, Na, Mg, Ca in leaf, growth, A, E	Grafted plants were less tolerant than nongrafted plants. Low water usage and low leaf chloride indicated tolerance.
Chlorophyll fluorescence and mineral nutrition in citrus leaves under salinity stress	F.J. Bleda, R. Madrid, A.L. García-Torres, Á. García-Lidón, and I. Porras	2011	2	‘Alemow’ (*Citrus macrophylla*) and ‘Cleopatra’ mandarin (*Citrus reticulata*)	‘Alemow’ (*C. macrophylla*) and ‘Cleopatra’ mandarin (*C. reticulata*)	0 mM, 25 mM, 50 mM	Fv/Fm, Na, Cl, P, K, N, Mg leaf	Grafted plants were slightly more tolerant than grafted plants. Higher Fv/Fm indicated tolerance.

A = net photosynthesis; E = transpiration rate; Fv/Fm = maximum quantum efficiency of Photosystem II; P = phosphorus; K = potassium; N = nitrogen; Cl = chloride; Na = sodium; Mg = magnesium; Ca = calcium.

papers assessed, leaf ion and root ion content were the most frequently measured parameters. These measurements indicate the concentration of ions taken up by the tree from the soil. Under identical salinity levels, trees that translocate higher ion concentrations to leaf tissues are generally more sensitive to salinity stress (Storey 1995). The tolerance of the tree is often linked to its ability to compartmentalize ions in the roots, preventing translocation to the leaves. If ions are not sequestered in the vacuoles, they accumulate in the cytoplasm, leading to cellular metabolic disruption, affecting ion uptake and essential metabolic pathways (Mansour 2023). For example, high chloride accumulation in leaf tissues has been shown to indicate less tolerance to salinity. ‘Carrizo citrange’ and ‘Cleopatra’ exhibited differing levels of Cl^- in leaves, with ‘Cleopatra’, the more tolerant cultivar, accumulating less Cl^- in the leaf tissues (Moya et al. 2002). Gas exchange measurements are also crucial for understanding the response of trees to both phases of salinity stress. As salt accumulation increases in the soil, osmotic stress reduces water uptake, resulting in stomata closure to conserve water (Safdar et al. 2019). This response decreases transpiration and limits CO_2 fixation, ultimately reducing photosynthesis (García-Sánchezet al. 2002a). The initial response to osmotic stress will affect the susceptibility of the tree to ion toxicity. This creates a trade-off: closing stomata limits water loss but also restricts gas exchange and photosynthesis, potentially affecting growth (Cabot et al. 2014). Conversely, trees that maintain higher transpiration rates under stress risk taking up more toxic ions, accelerating ion accumulation and damaging the photosynthetic system, leading to leaf senescence if the salinity threshold is exceeded (García-Sánchez et al. 2002b). Gas exchange measurements are valuable because they allow nondestructive assessment early in the experiment, providing continuous data on the tree response. Another important parameter is dry weight and overall tree growth, which indicate the tree’s ability to create organic matter or to grow under high-salinity conditions (Westlake 1963). Tolerant trees typically show sustained growth, which suggests successful adaptation to osmotic stress without excessive ion accumulation (Ben Yahmed et al. 2015). Other measurements, although less frequently used, include direct assessments of chlorophyll content and chlorophyll fluorescence (F_v/F_m). Both parameters are linked to the photosynthetic process; chlorophyll forms part of the Photosystem II (PSII) and it is involved in light absorption, whereas F_v/F_m is a performance indicator of PSII efficiency (Alemu 2020). Salinity causes an imbalance in cellular processes, leading to the generation of reactive oxygen species (ROS), which damage PSII and chlorophyll molecules, resulting in the reduction of both F_v/F_m and chlorophyll contents and eventually causing leaf chlorosis and senescence (Hasanuzzaman et al. 2021). Additional parameters, such as proline accumulation, fresh weight, stem diameter, symptoms in leaves, and water potential, were also measured in some studies. Proline acts as an osmolyte, helping to maintain cellular osmotic balance under salinity stress, and cultivars that accumulate more proline are often more tolerant. Fresh weight and stem diameter, dry weight, and height provide similar insights into the tree’s ability to respond to the stress and continue growing. Fresh weight is typically measured alongside dry weight to avoid misrepresenting tree biomass, and water content of the tree can be obtained with these two parameters. Stem diameter offers a useful, nondestructive measure of growth over time. Water potential assesses how trees adjust to osmotic stress. Salinity stress reduces the water potential, which trees compensate for by lowering the osmotic potential to maintain leaf turgor and water uptake. For example, Martínez-Cuenca et al. (2021) found that tolerant genotypes of ‘King’ mandarin exhibited better osmotic adjustment than sensitive genotypes like ‘Carrizo citrange’ under saline conditions. The parameters that were measured together in most of the reviewed papers were leaf ion content, growth, gas exchange, root ion content, and dry weight. This combination offers a comprehensive view of the response of the tree to salinity. Gas exchange measurements reflect the osmotic phase, whereas ion accumulation reflects the ion toxicity phase. Growth and dry weight provide a clear indication of the tree’s performance under stress, helping to identify tolerant cultivars. Choosing the right combination of measurements is essential for ensuring the efficiency and relevance of the experiment.

Q2: Is there an influence of grafting on salt tolerance evaluation?

In the context of salinity stress, the different responses between grafted and nongrafted trees, as well as the influence of the rootstock-scion combination, have been shown to significantly affect plant tolerance. Both studies that carried out an assessment of reciprocal grafts found different results. Moya et al. (2002) found that grafted trees were less tolerant than nongrafted trees by accumulating higher chloride concentrations in leaves. On the other hand, Bleda et al. (2011) found that grafted trees were more tolerant, showing a better growth performance under the salinity stress. Both studies used ‘Cleopatra’ as one of the cultivars, known as salinity tolerant; the difference was the selection of the second cultivar: ‘Carrizo’ in the first study, classified as salinity-sensitive, and ‘Alemow’ in the second study, classified as moderately tolerant (Pathania and Singh 2021). It suggests that the overall tolerance of the grafted combination is strengthened when both cultivars are tolerant, whereas combinations involving a susceptible cultivar may reduce salinity tolerance. When conducting studies that compare grafted and nongrafted trees, the tolerance of the cultivar must be known beforehand to avoid incorrect conclusions. This highlights the importance of the selection of rootstock-scion combinations when assessing salinity tolerance. Nongrafted trees

Table 2. Summary of studies on the effects of salinity on scion cultivars. Key findings from various studies examining the impact of salinity stress on different citrus scion-rootstock combinations.

Title	Author	Year	#Scions	Scion cultivar	Rootstock cultivar	Treatments (mM NaCl)	Parameters	Results
Effects of salinity on ionic content, water relations and gas exchange in citrus scion—rootstock combinations	M.H. Behboudian, E.T. Rokfalvy, and R.R. Walker	1985	3	'Valencia' orange (*Citrus sinensis*), 'Taylor' lemon (*Citrus lemon*), and 'Ellendale' tangor (*Citrus reticulata*)	Cleopatra mandarin (*C. reticulata*) and Rough lemon (*Citrus jambhiri*)	0 mM, 70 mM	Cl, Na, Kroot, leaf, stem. ψw, ψp, ψs, A, E, Chl	Different leaf Na accumulation, A, and E among the scions; Compared 0 mM different results were found for \Psi\w, \Psi\p, \Psi\s among scions.
Salt tolerance of 2 lemon scions measured by leaf chloride and sodium accumulation	M. Nieves, A. Cerda, and M. Botella	1991	2	'Verna' and 'Fino' lemon (*Citrus limon*)	Sour orange (*Citrus aurantium*) and Macrophylla (*Citrus macrophylla*)	2 mM, 40 mM, 80 mM	Cl and Na leaf, ψw, *gs*, Chl, proline, growth	Na and Cl leaf accumulation varied between scions. As well as chlorophyll and growth.
Effect of salinity on growth, ion content and CO2 assimilation rate in lemon varieties on different rootstocks	M.F. Garcia-Legaz, J.M. Ortiz, A. Garcia-Lidon, and A. Cerda	1993	3	'Fino', 'Verna', and 'Eureka' lemon (*C. limon*)	Sour orange (*C. aurantium*), Macrophylla (*C. macrophylla*), and Volkamer lemon (*Citrus volkameriana*)	5 mM, 25 mM, 50 mM	Cl and Na leaf, E, A, *gs*, Chl, growth	Na and Cl leaf accumulation varied between scions, as well as chlorophyll.
Role of rootstock and scion on root and leaf ion accumulation in lemon trees grown under saline conditions	A. García-Lidón, J.M. Ortiz, M.F. García-Legaz, and A. Cerdá	1998	3	'Fino', 'Verna', and 'Eureka' lemon (*C. limon*)	Sour orange (*C. aurantium*), Macrophylla (*C. macrophylla*), and Volkamer lemon (*C. volkameriana*)	5 mM, 50 mM	Cl, Na, K, N, Mg, Fe, Zn, Mn in root and leaf	Different Cl and Na concentration among scions.
Screening of citrus scion-rootstock combinations for tolerance to water salinity during seedling formation	M.E. Barbosa-Brito, P.D. Fernandes, H.R. Gheyi, L.A. Soares, W.S. Soares Filho, and J.F. Suassuna	2020	2	'Tahiti' acid lime (*Citrus latifolia*) and 'Star Ruby' grapefruit (*Citrus paradisi*)	12 citrus genotypes[i]	8 mM, 16 mM, 24 mM, 32 mM, 40 mM	Leaf, Stem, Root DW, survival	DW aerial parts and survival affected by Scion and Salinity Trt.
The response of three mandarin cultivars grafted on sour orange rootstock to salinity stress	S.M. Madani, S. Piri, and S. Sedaghathoor	2022	3	'Younesi', 'Clementine', and 'Yashar' mandarin (*Citrus reticulata*)	Sour orange (*C. aurantium*)	0 mM, 10 mM, 30 mM, 50 mM	Na, K leaf, root. Growth, Chl	Interaction between chlorophyll and scion cultivars.
Morphological, physiological, and molecular scion traits are determinant for salt-stress tolerance of grafted citrus plants	V. Vives-Peris, M.F. López-Climent, M. Moliner-Sabater, A. Gómez-Cadenas, and R.M. Pérez-Clemente	2023	2	'Navelina' orange (*C. sinensis*) and 'Oronules' mandarin (*Citrus clementina*)	Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*), and Macrophylla (*C. macrophylla*)	0 mM, 90 mM	MDA, Cl root and leaf, A, E, *gs*	Cl leaf, A, E, *gs* in scions grafted on CC.

[i] 'Santa Cruz Rangpur' lime (*Citrus limonia*); 'Sunki' mandarin (*Citrus sunki*); 'Swingle' citrumelo (*C. paradisi* × *P. trifoliata*); 'Sunki of Florida' mandarin; 'C25' citrange (*C. sinensis* × *P. trifoliata*); 'C13' cit-range; HTR - 069: trifoliate hybrid of 'Pera' sweet orange (*C.* ×*sinensis*) with 'Yuma' citrange; 'Troyer' citrange, 'Florida Rough' lemon (*Citrus jambhiri*); *P. trifoliata*; 'Rangpur' lime; 'Volkamer' lemon (*C. volkameriana*); 'Argentina' citrang.

Ψw = water potential; Ψp = pressure potential; Ψs = osmotic potential; A = net photosynthesis; E = transpiration rate; Chl = chlorophyll content; *gs* = stomatal conductance; Proline = proline content; N = nitrogen; Mg = magnesium; Fe = iron; Zn = zinc; Mn = manganese; Cl = chloride; Na = sodium; K = potassium; DW = dry weight; MDA = malondialdehyde.

are useful for initial evaluations, but they do not represent the real conditions of the commercial fields, where citrus is cultivated as grafted trees. Therefore, trials with grafted trees are essential for accurate assessments before making cultivar recommendations for specific growing regions.

Q3: What is the role of the scion cultivar on the salinity tolerance of the combination of scion and rootstock?

The resurgence of research after 2020 could be linked to renewed interest in improving citrus tolerance to abiotic stresses, including salinity, as climate change exacerbates salinity issues in many citrus-growing regions. The frequent use of specific scion cultivars—such as lemon (e.g., Fino and Eureka), sweet orange (e.g., Valencia), and mandarin (e.g., Clementina)—alongside rootstocks like Sour orange, Cleopatra, Rough lemon, and Carrizo citrange, suggests a strategic focus on commercially important and widely cultivated varieties. Notably, lemon scions appeared more often in these studies, which were carried out in Spain, which has the been the most active country in salinity research. Spain is also one of the largest producers of lemon among the countries assessed is this review, which may explain the predominance of lemon cultivars in salinity-related studies (FAO 2021).

Ion content, particularly chloride and sodium, is a critical parameter in the studies reviewed. The accumulation of these ions in leaves varied significantly across different scions, suggesting that ion accumulation is predominantly regulated by the scion cultivar. In all reviewed reports, scions with higher tolerance to salinity typically exhibited lower accumulation of Na^+ and Cl^- in their tissues, especially in leaves. This indicates that scions play a crucial role in moderating the uptake and distribution of these ions under saline conditions. Martínez-Alcántara et al. (2015) found that more tolerant cultivars tend to allocate higher concentrations of High-Affinity K^+ Transporter 1 (HKT1) transporters. HKT1 transporters are membrane proteins essential for transporting Na^+ ions across cell membranes, particularly under salinity stress. These transporters help maintain ion homeostasis by redistributing Na^+, thereby preventing it from reaching toxic levels in the leaves. In *Arabidopsis*, the deletion of the HKT1 gene led to severe Na^+ accumulation in leaves, reducing the tree's tolerance to salinity (Mäser et al. 2002). HKT1 transporters are expressed in the root stele and leaf vasculature, but their impact is more pronounced in regulating ion distribution in shoot tissues, preventing excessive sodium from reaching the leaves. Vives-Peris et al. (2023) found similar results for Cl^-, identifying two main transporters responsible for its translocation. Whether Na^+ or Cl^- impacts the plant depends on the cultivar's exclusion capacity (Snoussi et al. 2022). Further research is needed to determine whether the expression of these genes is more strongly associated with the scion than the rootstock.

Chlorophyll content was also affected by the scion, with a clear correlation between ion accumulation and chlorophyll degradation. Trees with higher Na^+ and Cl^- concentrations in their leaves had lower chlorophyll levels, suggesting a direct link between ion toxicity and the tree's ability to sustain photosynthesis. Salinity stress often leads to the production of ROS, which are known to degrade chlorophyll affecting to the tree's photosynthetic performance (Pan et al. 2021). Similar results were found in grapevines where the scion played a role in the inorganic ion accumulation, which influenced chlorophyll concentrations. In addition, Parihar et al. (2015) found that accumulation of the ions was correlated with the stomatal conductance and transpiration rates. Almond (*Prunus amygdalus*) scions grafted on the same rootstock differed in their tolerance to salinity by the accumulation of Na in their leaves (Momenpour et al. 2018). Li et al. (2022) found that scion also had an influence on the accumulation of toxic ions in the leaf in apple (*Malus domestica*) cultivars. On the contrary, Ferreira-Silva et al. (2010) found that rootstock had an influence on the ion accumulation and other physiological parameters in cashew (*Anacardium occidentale*) trees. Although most research has focused on the rootstock's role in salinity tolerance, these findings highlight the need for more comprehensive studies that consider the scion's contribution.

Conclusion

Salinity research in citrus has gained importance, with more publications in recent years, driven by climate change and water scarcity. Spain, the United States, and Brazil lead research efforts due to their significant citrus production and salinity challenges. Although arid and semiarid regions dominate salinity studies, humid areas like Florida also contribute due to water quality issues like saltwater intrusion.

In cultivar trials for salinity tolerance, key parameters commonly measured together include leaf and root ion concentrations, gas exchange, dry weight, and growth. To comprehensively assess salinity tolerance, we recommend incorporating measurements that capture both stages of stress: osmotic disruption and ion toxicity, along with tree development. This can be done through a combination of one-time (destructive) and continuous measurements, including

- Gas exchange: continuous, both stages.
- Ion concentration in plant tissues: end of the experiment, ion toxicity.
- Dry weight: end, overall tree performance.
- Growth: continuous, overall tree performance.
- Chlorophyll and/or fluorescence: end/continuous, photosynthetically performance.
- Water and osmotic potential: during experiment, osmotic disruption.

The addition of chlorophyll and/or chlorophyll fluorescence will provide insights on the capacity of the PSII to operate correctly under the stress being an important tolerance indicator. Also, water and osmotic potential will help to understand the capacity of the tree to adapt to the osmotic stress.

For initial cultivar assessments, nongrafted trees are ideal, particularly for breeding programs. However, grafted trees are recommended when the goal is to provide recommendations for commercial fields in specific regions.

Although rootstocks are often the primary focus, additional research on the influence of the scion and scion/rootstock combination on salinity tolerance is essential to optimize cultivar selection. The scion plays a role in salinity tolerance of the combination by influencing ion accumulation and distribution. Further research is needed to unveil the mechanisms behind it, with a focus on the potential role of the scion in the regulation of ion transporters (such as HKT1).

This systematic approach helped to quantify the information from each paper reviewed, presenting it in a way that allowed us to identify key data on salinity tolerance research in citrus. It provides an overview of the main study locations and assists other researchers by highlighting essential variables commonly used in these types of studies. In addition, it reveals gaps in knowledge regarding the role of grafted plants and the influence of the scion.

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