



Review

# Beneficial Microbes and Molecules for Mitigation of Soil Salinity in *Brassica* Species: A Review

Ines Petrić <sup>1</sup>, Dunja Šamec <sup>2,\*</sup>, Erna Karalija <sup>3</sup> and Branka Salopek-Sondi <sup>4</sup>

<sup>1</sup> Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10002 Zagreb, Croatia; ipetric@irb.hr

<sup>2</sup> Department of Food Technology, University Center Koprivnica, University North, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia

<sup>3</sup> Department for Biology, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina; erna.k@pmf.unsa.ba

<sup>4</sup> Department for Molecular Biology, Ruđer Bošković Institute, Bijenička 54, 10002 Zagreb, Croatia; salopek@irb.hr

\* Correspondence: dsamec@unin.hr

**Abstract:** Salt stress results from excessive salt accumulation in the soil can lead to a reduction in plant growth and yield. Due to climate change, in the future climatic pressures, changed precipitation cycles and increased temperature will increase the pressures on agriculture, including increasing severity of salt stress. *Brassica* species contains oilseed and vegetable crops with great economic importance. Advances in understanding the mechanisms of salt stress in *Brassica* plants have enabled the development of approaches to better induce plant defense mechanisms at the time of their occurrence through the use of beneficial microorganisms or molecules. Both endophytic and rhizospheric microbes contribute to the mitigation of abiotic stresses in *Brassica* plants by promoting the growth of their host under stress conditions. In this review we summarized so far reported microorganisms with beneficial effects on *Brassica* plants and their mode of action. Another approach in mitigating the harmful effect of soil salinity may involve the application of different molecules that are involved in the stress response of *Brassica* plants. We reviewed and summarized their potential mode of action, methods of application and pointed out further research directions.

**Keywords:** *Brassica*; priming; soil salinity; stress tolerance



**Citation:** Petrić, I.; Šamec, D.; Karalija, E.; Salopek-Sondi, B. Beneficial Microbes and Molecules for Mitigation of Soil Salinity in *Brassica* Species: A Review. *Soil Syst.* **2022**, *6*, 18. <https://doi.org/10.3390/soilsystems6010018>

Academic Editors: Thomas Baumgartl and Mandana Shaygan

Received: 16 December 2021

Accepted: 29 January 2022

Published: 3 February 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Salt stress is one of the abiotic stressors that can significantly affect soil fertility, stability, and biodiversity and consequently affect crop food production by reducing plant growth and yield [1]. Soils in coastal and arid and semi-arid areas may naturally have increased salinity. However, the bigger problem are soils that are not naturally saline but where unsustainable management practices cause increased salinity [2]. Unsustainable practices include [2] the use of irrigation water that may be of low-quality (for example containing sediment content from agriculture-induced erosion and chemical pollution from agriculture and industry), inappropriate irrigation methods, poor drainage, removal of deep-rooted vegetation leading to increased groundwater levels, mismanagement of agricultural soil amendments and fertilizers, and pumping of water that can cause salt problems not only in coastal plains but also in semi-arid and arid lands. The FAO (Food and Agriculture Organization of the United Nations) has recognized salinization as one of the most important problems at the global level for agricultural production, food security and sustainability in arid and semi-arid regions [2]. It has always been known that soil salinization affects crop production (reviewed in [3]), but this effect has become even more pronounced in recent years due to climate change. According to FAO [2], climate change is exacerbating the accumulation of salts in soils due to the expansion of drylands, water

scarcity, and sea-level rise, causing saltwater intrusion into coastal areas. Salts gradually accumulate in the soil, which starts as a small, hidden problem and develops into a serious degradation if not adequately addressed [2]. To cope with reduced crop production in affected areas, it is necessary to develop tools for sustainable management of salt-affected crop production by inducing salt tolerance in crops that can mitigate the harmful effects of soil salinization. In agriculture, sustainable practices mean good management of natural systems and resources, which includes building and maintaining healthy soils, judicious water use, minimizing pollution and promoting biodiversity. Therefore, increasing the salt stress tolerance of crops using sustainable practices is a challenging task.

*Brassica*, or cruciferous vegetables, is a genus of plants in the cabbage and mustard family (Brassicaceae). It contains oilseed and vegetable crops with a long history of agricultural use throughout the world. The most commonly grown and used *Brassica* plants include *B. oleracea* and *B. rapa*, which are used as vegetables. The seeds of *B. nigra*, *B. carinata* and *B. juncea* are also used as spices, and *B. napus*, *B. rapa*, *B. juncea* and *B. carinata* are mainly grown for the production of edible oils [4]. All developmental stages of *Brassica* species (from seed germination to adult plants), may be jeopardized by salinity stress, depending on salt stress intensity and duration, and the natural salt tolerance of the species [1]. It has been reported that the salt tolerance of Brassicaceae depends on the ploidy of their genome. Accordingly, amphitetraploid *Brassica* species, such as *B. juncea*, *B. carinata*, and *B. napus*, are relatively more tolerant to increased salinity compared to their diploid parents *B. campestris*, *B. nigra*, and *B. oleracea* [5]. However, there are some variations in salt tolerance within species and even within cultivars of the same species [6,7]. Salinity tolerance within *Brassica* genera exhibits significant inter- and intraspecific differences as evidenced by differential effects of salinity on total growth, electrolyte loss, proline accumulation,  $K^+/Na^+$  ratio, and accumulation of hormones and specialized metabolites [8–10]. Conventional breeding approaches, considered energy-efficient and economical, are leading to several new promising salt-tolerant amphidiploid species (reviewed by [8]). Possible approaches include genetic engineering, but legislation in some parts of the world limits the use of such plants.

On the other hand, the use of sustainable tools to increase salt tolerance in *Brassica* crops and mitigate the negative effects of soil salinity is globally recognized and played an important role in the “green revolution” that brought great benefits to agriculture and mankind [11]. Recent advances in understanding the mechanisms of salt stress in *Brassica* plants (reviewed by [1]) have enabled the development of approaches to better induce plant defense mechanisms at the time of their occurrence through the use of beneficial molecules or microorganisms. We are focused on plant biomolecules as well as naturally occurring microorganisms that enable development of eco-friendly strategies in mitigating the harmful effects of soil stress in agriculture. In this review, we have summarized literature data about beneficial microorganisms and molecules and their mechanisms of action, and various methods of their application (seed priming, foliar application, and root application), in *Brassica* plants.

## 2. Beneficial Microorganisms to Mitigate Effect of Salt Stress Tolerance on *Brassica* Plants

### 2.1. Plant–Microorganism Interactions in Salinity Stressed Environments

Complex interactions between plants and microorganisms, which have evolved evolutionarily between plants and their associated microbiomes, are increasingly recognized as critical determinants of plant health and growth [12,13]. Plant biomass production is directly influenced by microbial biodiversity and established symbiotic relationship, which contributes to plant nutrient uptake and other physiological processes. In extreme environments, these relationships are often exclusionary and based on even more complex mechanisms [14–16]. The system integrating soil exposed to salt stress, plants, and microorganisms represents a unique extreme environment that provides a venue for mutualistic relationships in which plant–microbe interactions play a crucial role in activating or stimulating different adaptive mechanisms for survival under salt stress [14,17]. This

phenomenon is also known as habitat-adapted symbiosis [18]. In this stressed environment, microbes enhance soil–water–plant relationships, manipulate phytohormonal signaling, and trigger other mechanisms that increase plant salt tolerance in an integrated manner, thereby maintaining plant fitness and health [19]. Unfortunately, salt stress strongly modulates physicochemical properties and reduces microbial activity, biomass and structure in and around plant roots [20–22]. This is believed to be due to their large area to volume ratio, high cell membrane permeability and rapid turnover rate, mainly due to osmotic pressure that shrinks the cells, resulting in water leakage from the microbial cells, thus slowing their growth [23].

In the study conducted by Andronov et al. [24] it was found that taxonomic composition of both bacterial and fungal microbial communities shifts with salinity gradients. Consequently, the vast majority of microbes fail to exert positive effects on plant growth under stressful conditions, giving an advantage to the population of salt stress-tolerant microbes [21,25,26]. These adapted microorganisms that inhabit the province of plants are selected from an enormous reservoir of microorganisms in the soil, including bacteria, archaea, and fungi, which eventually colonize plant roots (root microbiome), as well as shoots, leaves, flowers, and seeds (phytomicrobiome: endophytic (inside plant parts), epiphytic (on aboveground plant parts) and rhizospheric). When it comes to microbiomes that support plant growth under salt stress conditions, researchers often give preference to endophytic (living in root cells and tissues, abundance of  $10^4$ – $10^8$  bacterial cells/g of root tissues) or rhizospheric microbes (living in close proximity to plant roots, an abundance of  $10^6$ – $10^{10}$  bacterial cells/g soil) [19,27]. Endophytes are considered to have advantages over rhizospheric microbes as being protected from changes in the environmental parameters [19] and could therefore become a potentially better tool for the improvement of plant growth under stress conditions. In addition, fungi have been shown to be more susceptible to salt stress compared to bacteria, so the ratio of bacteria to fungi may be elevated in saline soils [22].

Salinity-tolerant microorganisms can be divided into two groups: halophiles (including halotolerant bacteria, weak halophiles and intense halophiles), which live in high salinity environments and require salt for growth, and halotolerant organisms, that can adapt to saline environments [22]. Halophiles harbor (halo)zymes, such as cellulases, xylanases, proteases, amylases, lipase and gelatinase, having polyextremophilic features, i.e., salt tolerance or salt-dependent catalytic properties [22]. These specific enzymes also represent important biological molecules, such as phytohormones and exopolysaccharides, being crucial in plant–microbiome interaction, in keeping the stability of the soil structures and water-holding capacity and are also useful for bioremediation in saline environments [22]. On the other hand, halotolerant microorganisms can also overcome the effect of salt by the accumulation of compatible solutes for osmoregulation, production of extracellular proteases and activation of  $\text{Na}^+/\text{H}^+$  antiporters [28]. Most bacteria survive under stress conditions by producing extracellular polymeric substances (EPS) that increase water retention and regulate diffusion of organic carbon sources [21]. The formation of biofilm is also one of the common features that enable microbes to adapt to salinity stress as they are supported by the formation of EPS.

Both endophytic and rhizospheric microbes contribute to mitigate abiotic stresses in plants with their potentially intrinsic metabolic and genetic capabilities [19,21,29]. The mechanism by which microbes promote the growth of their host under stress conditions can be in two ways: direct and indirect mechanisms [26]. In direct mechanisms, microbes can directly improve the reduced efficiency of plant nutrient uptake under salt-stressed conditions by providing to the plant already available soluble nutrients, such as phosphorus and potassium as well as mineral elements, by providing nitrogen via the N-fixation process or by increasing available iron in saline soils via the production of siderophores, while reducing sodium accumulation under saline conditions [19,21,26,29,30]. Microbes can also modulate hormonal imbalance in plant tissues by producing plant hormones such as auxins (IBA and IAA), cytokinins (CKs), abscisic acid (ABA), and gibberellins (GAs). These plant-

associated microbes are also capable of producing 1-aminocyclopropane-1-carboxylase (ACC) deaminase, which can reduce the level of deleterious ethylene known to accumulate in plants under stress conditions. Production of secondary metabolites such as extracellular polymeric substances (EPS) and various volatile organic compounds (VOC) by salt-tolerant microorganisms was found to directly modulate plant cellular responses through regulation of the *SOS1* gene, expression of stress-regulating genes, expression of high-affinity K<sup>+</sup> transporter (HKT1) genes, antioxidant protein genes, and ethylene biosynthesis [31]. EPS helps in establishing plant–microbe interactions by providing a microenvironment in which microbes can survive under stress conditions [21]. In indirect mechanisms, microbes act as biocontrol agents by containing populations of surrounding fungal and bacterial pathogens through the production of antibiotics and hydrolytic enzymes [32,33]. Importantly, microbes can also reduce the effects of salt stress in plants by accumulating organic osmolytes, proline, glycine betaine, or total phenolics, or by increasing gene expression of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), or glutathione reductase (GR) [19,26]. These compounds are extremely important plant allies in osmotic regulation, stabilization of cellular components, and scavengers of reactive oxygen species (ROS). Unfortunately, osmolyte production costs a considerable amount of energy and requires a massive C-skeleton, resulting in reduced activity and growth [22]. Abiotic stress is the primary cause for the generation of ROS in plants, the toxicity of which affects the structure and function of plant biomolecules [34]. Application of bacterial inoculum in plants subjected to salt stress has also been shown to increase the activity of defence-related enzymes such as polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) [26] or induce the expression of salt stress-responsive genes such as *DREB2b*, *RD29A*, *RD29B* and *RAB18* [21].

Various studies have shown that all these different mechanisms aimed at altering plant physiology to better tolerate stress conditions are regulated by different microorganisms, including members of the genera *Rhizobium*, *Trichoderma*, *Pseudomonas*, *Acinetobacter*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Dietzia*, *Klebsiella*, *Streptomyces*, *Serratia*, *Sphingomonas*, *Enterobacter* and *Burkholderia* etc. [19,21,29]. Although different microbes have been associated with different mechanisms in supporting plant growth under saline conditions, it is now increasingly clear that the growth-promoting abilities are likely the result of an additive contribution of many taxa within the soil microbiome and not just from one particular species. Researchers are increasingly focusing on the use of growth-promoting bacteria (PGPB) and/or the microbiome as a biological tool to mitigate salt stress in plants for sustainable agriculture. However, there is a need to better explore the hidden diversity of these microbes to understand their physiological and functional properties and to exploit their potential.

## 2.2. Microorganism with the Ability to Enhance Salinity Tolerance in Brassica Plants

The literature review on the possible beneficial effects of different microbes on the growth of *Brassica* species under salt-stressed conditions confirmed the positive relationships between microorganisms and *Brassica* plants (Table 1.), resulting from different mutualistic mechanisms, as mentioned earlier. Out of different *Brassica* species majority of the retrieved studies were found to be focused on *B. napus* with only a few dealing with *B. juncea*, *B. campestris* and *B. rapa* species. In general, studies followed the beneficial effects of the inoculation of *Brassica* plants or seeds with different bacterial or fungal species, designated as plant-growth promoting (PGP) microorganisms or adapted halotolerant microorganisms.

**Table 1.** Beneficial microorganisms in mitigating salt stress and its mechanism of action in *Brassica* species.

Identity of the Beneficial Microorganism	Host Plant	Mechanism for the Salinity Mitigation
<i>Enterobacter cloacae</i> HSNJ4 [35]		Increase in indole-3-acetic acid
<i>Pseudomonas putida</i> UW4 [36]		Higher expression of photosynthesis and anti-oxidative processes; transportation across membranes; pathogenesis-related proteins
<i>Azotobacter chroococcum</i> NBRC, <i>Alcaligenes faecalis</i> NBRC 13111 [37]	<i>B. napus</i>	Enhanced growth and photosynthesis, higher levels of soluble sugars and proteins as well as antioxidant enzymes, decline in proline, malondialdehyde (MDA), and hydrogen peroxide, increase in N, K, Ca and Mg uptake
<i>Arthrobacter globiformis</i> CD [38]		Increase in phenolic compounds and proline; enhanced activities of phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD)
<i>Pseudomonas fluorescens</i> FY32 [39]		Increase in plant dry weight and length of roots; increase in proteins related to energy metabolism and cell division
<i>Trichoderma harzianum</i> T22 [40]		Improved uptake of nutrients; aggregation of osmolytes and antioxidants; reduced uptake of NaCl
<i>Pseudomonas argentinensis</i> HMM5, <i>Pseudomonas azotoformans</i> JMM15 [41]	<i>B. juncea</i>	Increase in root, shoot and plant dry weight
<i>Brevibacterium iodinum</i> RS16, <i>Micrococcus yunnanensis</i> RS222, <i>Bacillus aryabhatai</i> RS341, <i>Bacillus licheniformis</i> RS656 [42]	<i>B. campestris</i>	Increased vigor index, fresh weight and growth hormones; production of stress alleviating enzymes
<i>Bacillus subtilis</i> GOT9 [43]		Improvement of the growth of aerial parts
<i>Herbaspirillum</i> sp. GW103 [44]	<i>B. rapa</i>	Production of auxin, siderophore, and 1- aminocyclopropane-1-carboxylic acid deaminase; increase in K <sup>+</sup> /Na <sup>+</sup> ratio in roots; increase of plant biomass

In the study by Li et al. [35], it was found that canola (*B. napus*) plants inoculated with the halotolerant bacterium *Enterobacter cloacae* HSNJ4 became more salt-tolerant, and the authors linked this new trait to the increase in endogenous indole-3-acetic acid (IAA) concentration. Cheng et al. [36], in their work on the same *Brassica* species, compared the differences in proteome profiles of plant shoots and roots in control plants and those inoculated with a different plant growth-promoting bacterium (PGPB) *Pseudomonas putida* UW4 under salt stress. The effects of the bacteria included higher expression of a number of different processes in the plant, including photosynthesis, antioxidant processes, transport across membrane carriers, and proteins associated with pathogenesis. Salinity tolerance of *B. napus* was also recently studied by Latef et al. [37]. They tested the possible role of the two PGPB *Azotobacter chroococcum* NBRC and *Alcaligenes faecalis* NBRC 13111 on the growth attributes, photosynthetic pigments, osmolytes, oxidative stress and antioxidant enzyme activities of the tested plants. The positive correlations obtained indicate that co-inoculation with selected beneficial bacteria can be considered an excellent tool to combat saline soil problems. To improve plant tolerance to (salt) stress, beneficial microbes can also be inoculated onto plant seeds [45]. This method, called biopriming, was tested on *B. napus* in the work of Stassionos et al. [38]. The authors tested the potential beneficial effects of the halotolerant strain *Arthrobacter globiformis* CD on the salt stress response of the oilseed rape plant by inoculating the selected bacteria into the seeds of cultivars with different salt tolerance. The results showed a better stress response of the inoculated plants to high salt concentrations, especially in the sensitive cultivar, with a significant increase in the content of phenolic compounds and increased enzymatic activity of phenylalanine ammonia lyase (PAL) and superoxide dismutase (SOD). The concentration of the osmolyte proline was also significantly increased in the inoculated plants compared with the noninoculated plants. With the aim of also better understanding the role of PGPB in promoting growth of oilseed rape cultivars with different salt sensitivity (salt-sensitive Sarigol and salt-tolerant Hyola308 cultivars) under salt-stressed conditions, Banaei-Asl et al. [39] inoculated plants with PGPB *Pseudomonas fluorescens* FY32. Positive effects were observed on plant morphology by increasing dry weight and length of the canola roots as well as on protein profiles of roots. The results suggest that tolerance to salt stress is increased by an increase in proteins related to energy metabolism and cell division (proteins related to amino acid metabolism and the tricarboxylic acid cycle).

For *B. juncea*, it has also been shown that treatment with beneficial fungi or bacteria results in favorable growth. Inoculation with the fungus *Trichoderma harzianum* T22, known as plant growth promoter fungi (PGPF) and biocontrol agent, improved plant oil content, plant uptake of essential nutrients, enhanced aggregation of osmolytes and antioxidants and reduced NaCl uptake [40]. In another study [41], co-inoculation of *B. juncea* grown under salt stress with halotolerant bacteria *Pseudomonas argentinensis* HMM57 and *Pseudomonas azotoformans* JMM15 resulted in significant increase in the root, shoot and dry weight of the plants. Due to the importance of biofilm formation under stress conditions, Hong et al. [42] investigated the effect of salinity fluctuations on exocellular polysaccharide (EPS) production, biofilm formation and flocculation in their work. This study was conducted on the species *B. campestris* by inoculating the plants with four different halotolerant bacteria: *Brevibacterium iodinum* RS16, *Micrococcus yunnanensis* RS222, *Bacillus aryabhatai* RS341, *B. licheniformis* RS656. The contingency analysis showed that the tested parameters were strain-dependent. It was clear that the correlation with salinity was positive, at least for the first two parameters (EPS production, biofilm formation). All halotolerant strains promoted the growth of *B. campestris*, as evidenced by the increased vigor index and fresh weight regardless of salinity. Finally, there are several studies on cabbage species. It was shown that the addition of *Bacillus subtilis* (strain GOT9) during the growth of cabbage *B. campestris* L. ssp. *pekinensis* under salt-stressed conditions improved to some extent both the drought and salt tolerance of the host plant [43]. Although no significant change was observed between controls and GOT9-treated plants, growth of aboveground parts was slightly improved in the presence of GOT9.

In research on Chinese cabbage (*B. rapa* L. ssp. *pekinensis*) [44], the addition of the strain *Herbaspirillum* sp. GW103, isolated from the rhizosphere of *Phragmites australis*, was shown to alleviate salt stress in the host plant. This occurred through the release of plant-promoting factors such as auxin, siderophores and 1-aminocyclopropane-1-carboxylic acid deaminase. In addition, the authors observed an increase in the  $K^+/Na^+$  ratio in the samples treated with the selected strain, resulting in an increase in plant biomass. All these research studies mentioned above suggest that various *Brassica* species are capable of establishing mutualistic relationships with their microbiome, which could eventually help them cope with the stress caused by increased salinity. However, much more interdisciplinary research is needed to fully characterize this positive relationship, with the ultimate goal of utilizing beneficial and/or salt-tolerant microbes as a biotic resource that could minimize the damage caused to *Brassica* plants by induced salt stress.

### 3. Beneficial Molecules to Mitigate Effect of Salt Stress Tolerance on *Brassica* Plants

#### 3.1. Molecular Response of Plants to Salt Stress

Increased soil salinity triggers complex abiotic stress in plants, including osmotic stress caused by decreased water uptake, toxicity stress caused by excessive concentration of  $Na^+$  and  $Cl^-$  ions, and oxidative stress caused mainly by increased accumulation of reactive oxygen species (ROS) [46]. Salinity stress causes disturbances in physiological, biochemical and metabolic processes important for plant growth and development. It can cause changes in gene transcription, primary and secondary metabolism, undesirable accumulation of ROS, impairment of ion homeostasis, alteration of membrane permeability, reduction in photosynthetic efficiency, inhibition of growth and reduction in biomass production and consequently lead to a significant reduction in crop yield [47]. Salt tolerance is achieved through regulation of  $Na^+$  and  $K^+$  transport, accumulation of various osmoprotectants, reduction of ROS toxicity through production and accumulation of antioxidants, and maintenance of hormone homeostasis that allow proper growth and development under adverse environmental conditions [48].

##### 3.1.1. Proline

Proline has been described as a reliable stress marker under salt stress, suggesting a positive correlation between proline accumulation and plant stress [49]. It can act as an osmoprotectant, but is also known as a metal chelator, antioxidant defense molecule, and signaling molecule. A positive correlation between proline content and stress intensity was found in several *Brassica* species [7,50]. However, the correlation between proline accumulation and salinity tolerance is controversial. Comparative studies between *B. oleracea* varieties under salt stress indicate that higher proline content is associated with more sensitive species/varieties [7]. A positive correlation between proline accumulation and salt tolerance was found in a comparative study of different *B. juncea* cultivars [50] and in rapeseed (*B. napus*) subjected to salt stress [51]. Thus, proline accumulation and its correlation with salt stress tolerance appear to be species- or even cultivar-specific in *Brassicaceae*.

##### 3.1.2. K/Na Ratio

Tolerance to salt stress is mainly related to the ability of plants to maintain  $K^+$  uptake despite competition from  $Na^+$ . The prevention of ion toxicity under salt stress is driven by the compartmentalization of sodium ( $Na^+$ ) ions in vacuoles and the ability to maintain high  $K^+/Na^+$  ratios [52]. Salt-tolerant brassicas (kale and white cabbage) have been shown to have higher  $K^+/Na^+$  ratios in their leaves than salt-sensitive Chinese cabbage cultivars [7]. The observations of higher  $K^+/Na^+$  ratios in leaves and lower ratios in roots of white cabbage and kale suggest that the control of  $Na^+$  transport from roots to leaves is more efficient in these cultivars. Studies show that plasma membrane (PM)  $H^+$ -ATPases and tonoplast (TP)  $H^+$ -ATPases/ $H^+$ -PPases,  $K^+$  transporters,  $Na^+/H^+$  exchangers (NHX), and the antiporter, Salt Overly Sensitive 1 (SOS1), are important components in the process of transporting  $K^+$  and  $Na^+$  and maintaining a favorable  $K^+/Na^+$  ratio [53].

### 3.1.3. Plant Hormones

Plant hormones are small bioactive molecules that act as powerful signals. They play a key role in the ability of plants to adapt to different environmental conditions by regulating development, growth and nutrient partitioning. They are involved in signal transduction by interacting with ROS and orchestrating the plant response to abiotic stresses by causing changes in transcriptomic, metabolic and proteomic networks that lead to plant acclimation and survival [54]. Salt stress-induced growth reduction has been shown to be associated with an imbalance in plant hormones [55]. Plant response to salt stress is highly regulated by several interactions, including hormone/ROS signaling, the excessive salt-sensitive signaling pathway, or MAPK signaling cascades leading to the expression of stress-related genes [56]. In addition to growth-promoting hormones such as auxin, cytokinins (CKs), gibberellin (GA), brassinosteroids (BRs), and strigolactones (SLs), plant stress hormones such as ABA, SA, JA, and ethylene are highly involved in plant stress response and tolerance to salinity (reviewed by [57]).

The plant root is the first organ to be confronted with increased salinity in the soil. The plant hormone auxin, which controls root development and architecture, plays an important role in root response to salt stress. Root growth inhibited by salt has been shown to be associated with a significant reduction in IAA during short-term stress [58]. The auxin metabolome was consistent with gene expressions, with the most striking changes observed in the transcripts of *YUC* (YUCCA flavin monooxygenases), *GH3* (auxin amidosynthetases), and *UGT* (uridine diphosphate glycosyltransferase) genes, suggesting a disruption of auxin biosynthesis, but particularly in the processes of amide and ester conjugation. In addition to metabolism, polar auxin transport has also been shown to be involved in reduced auxin accumulation in the root during salt stress [58–60]. Under salt stress, IAA showed a tendency to increase in *B. rapa* seedlings [61]. Moreover, bacteria secreting IAA, such as the root colonizing halotolerant bacterium *B. licheniformis* HSW-16 and selected rhizospheric *Bacillus* spp. have been reported to be able to mitigate the damage caused by elevated salinity [62]. Cytokinins (CKs) are plant hormones that are highly involved in the regulation of plant growth and development, but also in stress responses. Published data suggest that the role of CKs in salt stress can be contradictory and depends on plant species, applied salt concentration, and plant growth stage [63]. Reduced growth under salt stress is also associated with reduced gibberellic acid GA levels in plants [64]. Brassinosteroids (BRs) and strigolactones (SLs) are relatively new groups of phytohormones that play a central role in regulating plant growth and development and stress response [65,66]. Comparative studies on three brassicas with different salt tolerance showed a negative correlation between the content of endogenous active BRs and salt tolerance [7]. The high accumulation of brassinolide (BL) in the salt-sensitive cultivar correlated with its stronger inhibition of root growth observed in the root growth bioassay.

While endogenous growth-promoting phytohormones generally decrease under salinity conditions, the opposite is reported for most stress hormones. The ethylene precursor 1-aminocyclopropane-1-carboxylic acid accumulated and abscisic acid (ABA) increase under salinity [55]. ABA is the major player in response to salt stress by mediating a decrease in stomatal conductance, reducing the influx of toxic ions into aboveground plant parts, enhancing photosynthesis and osmolyte content, and reducing ROS-mediated toxicity. It is known to regulate salinity-responsive genes [64]. Significant increases in ABA were observed in a salt-tolerant *Brassica* cultivars [7] under elevated salinity conditions. JAs have also been reported to be involved in the response to salt stress and its level could decrease in some plant species (see reviews [67,68] including *B. rapa* seedlings [61], suggesting that the accumulation of jasmonic acid (JA)s in some species might be protective against salt stress. As there are controversial reports on JAs content and responses to salt stress, it is not possible to establish a general correlation between endogenous JAs content and salt tolerance [67]. In addition to its involvement in biotic stresses, salicylic acid (SA) also plays an important role in plant salt tolerance. Depending on the concentration of applied SA, the plant species and the developmental stage of the plants, contradictory effects of



exogenously applied SA on plants exposed to salt stress have been reported. In general, low concentrations of SA can enhance abiotic stress tolerance, while high concentrations induce oxidative stress, resulting in increased sensitivity to abiotic stress [69,70]. The protective role of SA under salt stress on stomatal conductance, net photosynthetic rate, and transpiration, as well as activation of antioxidant systems, was found [71–73]. The relationships between endogenous SA level and salinity tolerance are also controversial. The level of endogenous SA was found to decrease in *B.rapa* seedlings [61], while the level of SA increased in adult plants [73] under salt stress compared to controls. Ethylene, the gaseous plant hormone, modulates responses to salt stress by maintaining homeostasis of  $\text{Na}^+/\text{K}^+$ , nutrients, and ROS by inducing antioxidant defense, stomatal conductance, water use efficiency, and osmotic adjustment [74]. More or less, no stress hormone acts alone on the response to salt stress. There are complicated interactions and signaling networks among different plant hormones involved in salt stress response and tolerance [54].

#### 3.1.4. Polyphenolic Compounds

Phenolic compounds are usually potent antioxidants involved in combating ROS and protecting plants from abiotic stresses [75]. A positive correlation between salt tolerance and phenolic compound content has been observed in many halophytes (salt-tolerant species) [76]. Salt tolerance has been reported to correlate with the content of specific metabolites, including phenolic compounds, in different plant species and cultivars [75]. Comparative studies on three *Brassica* plants (kale, white cabbage, and Chinese cabbage) showed a positive correlation between phenolic acids and salt tolerance. The more tolerant kale and white cabbage accumulated significantly higher levels of phenolic acids, especially hydroxycinnamic acids (mainly sinapic acid (SiA), ferulic acid (FA), caffeic acid (CaA), 4-coumaric acid (pCoA)) and suffered less metabolic disturbance under salt stress than the more sensitive Chinese cabbage [9].

### 3.2. Molecules Used to Enhance Salt Tolerance in Brassica Plants

Knowledge of the molecular mechanisms that plants use to protect themselves from stress has enabled the development of application techniques that promote rapid plant response to stress, thereby mitigating the effects of stress. Beneficial molecules can be applied during different stages of growth, depending on the molecules used and the desired effect. The application methods can be seed priming, foliar application and root application.

#### 3.2.1. Seed Priming

A commonly used method is the application of different molecules at the seed level, called seed priming. The principle of seed priming is based on controlled hydration of the seeds to absorb water and trigger pre-germinative metabolism, but without the formation of radicles (which is ensured by desiccation of the seeds before radicle emergence). Pre-germinative metabolism involves the activation of enzymes [77], the build-up of metabolites [78] and other processes that contribute to the reduction of lag time during germination [79] and other beneficial effects on seed performance. Through this process, seeds are in a “primed state” that stores this short pulse and provides for more robust and resilient plants [80–82]. Controlled hydration during the reversible stages of germination leads to metabolic changes and, in some cases, changes in gene expression patterns [82], resulting in improved vigor, germination rate, growth and stress tolerance [78,80,82]. Priming of seeds includes different types depending on the method/compounds used: hydropriming (water), halopriming (inorganic salt), osmopriming (osmotic solution), hormonal priming (phytohormones), biopriming (microorganisms), priming with nanoparticles, etc. In general, halopriming (i.e., NaCl,  $\text{KNO}_3$ ,  $\text{CaCl}_2$  and  $\text{CaSO}_4$ ) and osmopriming (sugars, polyethylene glycol, glycerol, sorbitol, mannitol, proline) have been most commonly used to improve salt stress tolerance in various plants [83], but other species are also under investigation.

Seed priming methods for improving salt stress tolerance of *Brassica* plants are shown in Table 2. As can be seen, halopriming with NaCl and KCl is a common method for *Brassica*

plants and increases salt stress tolerance and production of specific metabolites, which may have a positive effect on the nutritional value of the shoots [84–86]. Research has shown that osmopriming with PEG, ZnSO<sub>4</sub> and CuSO<sub>4</sub> or KNO<sub>3</sub> solution with osmotic potential can be beneficial for *Brassica* plants as it improves germination and seedling growth under salt stress [87–89]. Priming seeds with gibberellic acid, a plant hormone, helps to mitigate the deleterious effects of salt on white cabbage (*B. oleracea* var. *capitata*) by reducing cellular damage under both control and NaCl stress conditions [90]. However, the positive effect of gibberellic acid under salt stress on *B. napus* was found only for the salt-tolerant cultivar, while it showed no effect in the salt-sensitive cultivar [91]. In addition to gibberellic acid, NO and triacntanol, with growth-regulating activity, showed promising effects in mitigating the adverse effects of salt stress on *Brassica* plants [92,93]. Hydropriming with distilled water increases germination and early seedling growth under salt stress in *B. rapa* subsp. *pekinensis* [94]. In a direct comparison of seed hydropriming with selenium nanoparticles (SeNPs) priming for protection against salt stress in *B. napus*, nanoparticles showed better mitigating effect [95]. Nanoparticles enhanced seed germination and improved antioxidant activities by modulating the expression levels of ABA and GA genes [95]. As per the literature, seed priming can be effectively used to improve *Brassica* seed performance, seedling vigor and resistance, tolerance to salt stress, and nutritional value of plants. However, the efficiency of seed priming may depend on the *Brassica* cultivar and the conditions to which the primed seed is exposed, as well as the type of seed priming used. This technique needs further research as it is a relatively simple method of improving seed performance that can be used in crop production and easily applied by farmers. Field trials and trials on fully matured plants are most needed because, as shown in Table 2, most experiments are conducted in laboratories and on seedlings or young plants.

### 3.2.2. Foliar Application

In addition to seed priming, plants at an advanced stage of development can be treated with beneficial molecules. Any application of molecules/chemicals prior to stress exposure can be referred to as chemical priming, where different molecules are applied to contribute to the plant's stress response. Foliar application (by spraying) has been shown to avoid the problem of leaching in the soil and elicit a rapid response in the plant. It is the method of choice for applying growth regulators or other mediators of the stress response.

Foliar application allows the treatment of plants at advanced stages of growth and monitoring of the effect on adult plants. Table 3 summarizes the molecules used for foliar application on *Brassica* plants. The effect of foliar application of proline, a known osmoprotectant involved in salt stress response, on *B. juncea* showed a limited protective effect in a study by Wani et al. [96]. In another study by the same group on the same species, the simultaneous application of epibrassinolide, a plant hormone belonging to the brassinosteroid class, and proline completely neutralized the negative effects of salt at 78 mM or 117 mM, while the treatment partially neutralized the effects of the highest salt concentration of 156 mM, through the upregulation of the antioxidant system [97]. Foliar application of epibrassinolide, alone or in combination with silicone, also mitigated salt stress-induced damage in *B. juncea* [98].

So far, the most studied molecules applied through the leaves to mitigate the effects of salt stress in *Brassica* plants are salicylic acid (SA). It showed a promising effect on *B. napus* [99,100] and *B. juncea* [101]. The combination of SA and 28-homobrassinolide can completely offset the negative effects of salt stress on *B. juncea* [102]. However, the effect of SA on *B. juncea* may depend on the cultivar. Syeed et al. [101] reported that the effect of SA was more pronounced in salt-tolerant cultivars. In contrast, the beneficial effect of SA foliar application was not dependent on cultivar as found by Husen et al. [103] in *B. carinata*, an amphidiploid plant between *B. oleracea* and *B. nigra*. Vegetables from the brassica group, such as choysum (*B. parachinensis*) [104] and Chinese cabbage (*B. rapa* L. ssp. *pekinensis*) [73] also suffer less damage from salt stress after application of SA. In a research report by Linić et al. [73], ferullic acid resulted in better improvement of salt tolerance in

Chinese cabbage compared to SA, suggesting that other phenolic acids may also have a beneficial effect on *Brassica* plants under salt stress. Methyl jasmonate, a volatile compound involved in various physiological processes in plants, counteracted the inhibitory effects of NaCl by increasing relative water content, soluble sugar content, and photosynthetic rate in an experiment by Ahmadi et al. [105] on *B. napus*. Similarly, inhibition of the negative effects of salinity showed 5-aminolevulinic acid (ALA), an important biosynthetic precursor of tetrapyrroles essential for plant growth and adaptation to stress environments [106]. Foliar application has great potential as a method of applying growth regulators that can help with salt stress tolerance. However, additional experiments are needed in *Brassica* plants, taking into account different developmental stages during spraying and *Brassica* varieties.

### 3.2.3. Root Application

The third way of applying beneficial molecules is to the root system, by introducing molecules into the soil or media in which plants grow. Here it is important to note that such application can affect not only the plants but also the rhizosphere microbiome, whose role in responding to salt stress we discussed above. Most research focuses only on the plant response and more research is needed that comparatively examines the microbiota and the plants.

Information on molecules used on *Brassica* plants is summarised in Table 4. This type of application is appropriate for hydroponically grown plants, and most research on *Brassica* plants is conducted using *B. napus* as a model plant. Molecules that have a positive effect on salt stress tolerance in *B. napus* are strigolactone GR24, poly( $\gamma$ -glutamic acid), silicone, melatonin, and serotonin [107–112]. Most of these molecules alleviate the oxidative stress caused by salinity and help to mitigate the negative effects. Thiourea, an ROS scavenger, also has a similar effect on *B. juncea* [113].

Although molecules have shown promise, there are no data from field trials that provide more realistic information, and such studies are certainly needed in the future.

**Table 2.** List of molecules, experimental design and results of a study on use of seed priming for salt stress tolerance improvement on *Brassica* plants.

Molecules	Brassica Plant	Experimental Design	Results
NaCl [84]	<i>B. napus</i>	Seed primed with 14 dS m <sup>-1</sup> NaCl solution for 24 h at 20 °C. Plants grown in the greenhouse and watered with five different NaCl solutions (0.4 (control), 4, 8, 12 and 16 dSm <sup>-1</sup> ), for a period of 3 weeks.	Increased salt tolerance NaCl priming enhanced proline accumulation and prevented toxic and nutrient deficiency effects of salinity due to less Na <sup>+</sup> but more K <sup>+</sup> and Ca <sup>2+</sup> accumulation.
KCl NaCl [85]	<i>B. oleracea</i> var. <i>capitata</i>	Seeds were soaked in KCl 50 mmol·L <sup>-1</sup> ; NaCl 150 mmol·L <sup>-1</sup> for 10 h in a controlled dark chamber at 25 °C Seedlings were sprayed with 150 mM NaCl.	Increase of secondary metabolite production, KCl seed priming adding nutritional value to cabbage sprouts
NaCl KNO <sub>3</sub> [86]	<i>B. napus</i>	Seed priming with 1% NaCl or 3% KNO <sub>3</sub> 48 h at 25 °C in darkness. Seeds cultivated in Petri dishes containing filter paper soaked with water, NaCl or CaCl <sub>2</sub> solutions 2:1 molar ratio of NaCl and CaCl <sub>2</sub> .	Increase in germination percentage and index, seedling fresh and dry weight probably due to repair mechanisms during imbibition. KNO <sub>3</sub> had stronger stimulation of germination percentage.
Polyethylene glycol (PEG) [88]	<i>B. napus</i>	Seed priming with PEG solution with osmotic potential 1.2 MPa for 7 days in the darkness at 25 °C. Seeds cultivated in Petri dishes containing filter paper soaked with NaCl solution (100 mM NaCl) or water (control).	Improved germination and seedling vigour under salt conditions. Increase of gene expression for proline biosynthesis (P5CSA and P5CSA)—up to 279% higher expression of P5CSA under NaCl stress.
ZnSO <sub>4</sub> and CuSO <sub>4</sub> [87]	<i>B. rapa</i>	Seed priming with ZnSO <sub>4</sub> and CuSO <sub>4</sub> . Seed germination under different levels of NaCl (60, 90 and 120 mM)	Increase in germination and seedling growth in both salinity levels as well as in non-saline conditions.
KNO <sub>3</sub> [89]	<i>B. napus</i>	Seed priming with KNO <sub>3</sub> solution with osmotic potential −1.0 MPa one day at 30 °C. Pots placed in a greenhouse and irrigated with NaCl with different levels of salinity (0.2, 5, 10, 15 and 20 dS m <sup>-1</sup> )	Improvement of seedling growth, development, and establishment of primed plants under salinity stress.
GA <sub>3</sub> [91]	<i>B. napus</i> var. <i>oleifera</i>	Seed priming with GA <sub>3</sub> (1 mM), solution for 12 h. Seeds cultivated in Petri dishes containing filter paper soaked with water, seedlings transferred to chromatography paper strips dipped in 100- and 250-mM NaCl	GA <sub>3</sub> improved salt tolerance in salt-tolerant cultivar with no effect on salt-sensitive cultivar
gibberellic acid (GA <sub>3</sub> ) [90]	<i>B. oleracea</i> var. <i>capitata</i>	Seeds were soaked for 10 h in 0, 100, 150 or 200 mg/l GA <sub>3</sub> . Plants were grown in a greenhouse. Salt treatments were started 15 DAS by adding 0, 50, 100 or 150 mM NaCl to the nutrient solutions, and plants were harvested 30 DAS.	GA <sub>3</sub> alleviated the harmful effect of salt stress on cabbage in terms of fresh and dry weights. Plants grown from GA <sub>3</sub> -primed seeds suffered lower cellular injury both under control conditions and under NaCl stress.
distilled water [94]	<i>B. rapa</i> ssp. <i>pekinensis</i>	Seed priming with distilled water 10 h at 20 °C in darkness Seeds cultivated in Petri dishes containing filter paper soaked with NaCl (50, 100, 150, 200 and 250 mM).	Increase in germination traits and early seedling growth under salt stress
NO [92]	<i>B. oleracea</i>	Seeds were soaked in 0.02 mM NO for 12 h. Broccoli seedlings were subjected to saline stress (NaCl) in Hoagland's solution enriched with all essential nutrients	NO was effective in increasing plant growth, chlorophyll pigments, glycine betaine, proline and oxidative defense system

Table 2. Cont.

Molecules	Brassica Plant	Experimental Design	Results
triacontanol [93]	<i>B. napus</i> L.	Seeds were soaked in different levels of TRIA (i.e., 0, 0.5, 1.0 mg L <sup>-1</sup> ) for 12 h and salt stress (100 and 150 mM) was applied on 56-days-old plants and data recorded three weeks after treatment	Pre-sowing seed treatment with TRIA increased shoot fresh weight, number of seeds per plant, photosynthetic rate, transpiration rate, ratio of chlorophyll a/b, q, electron transport rate, shoot and root K contents, and free proline and glycine betaine contents of canola plants at various TRIA levels under no-saline or saline conditions.
Selenium nanoparticles (SeNPs) [95]	<i>B. napus</i> cultivars	Seed priming with 10 mM sodium selenite (Se (IV)) for 72 h, ddH <sub>2</sub> O for hydropriming. Seeds cultivated in plastic boxes with filter paper soaked with 150 mM NaCl.	Nanoparticles elevated seed germination and improved antioxidant activities by modulating expression levels of ABA and GA genes

Table 3. List of foliar treatments with molecules and their beneficial effect on *Brassica* plants under salt stress.

Aget	Plant	Experimental Design	Results
proline [96]	<i>B. juncea</i>	At 29 days after sowing, plants were sprayed with either 20 mM proline or water in the presence or absence of NaCl stress.	Exogenous application of proline counteracted the effects of salt stress in Varuna only, by increasing the antioxidative capacity of the plants. Moreover, proline was not effective in alleviating the detrimental effects of higher salt concentrations on the studied parameters.
Epibrassinolide and proline [97]	<i>B. juncea</i> cv. Varuna and cv. RH-30	The leaves of were sprayed thrice at an interval of 10 min with 20 mM proline at 28 DAS and/or 10–8 M EBL at 29 DAS. Plants were sampled at 60 DAS for stress parameters and at mature stage (120 DAS) for yield characteristics.	Exogenous application of EBL with proline completely neutralised the adverse effects of salt at 78 mM or 117 mM, whereas the treatment partially neutralised the impact of highest salt concentration of 156 mM, through the upregulation of the antioxidant system.
Silicon (Si) 24-Epibrassinolide (EBL) [98]	<i>B. juncea</i>	Seeds of <i>B. juncea</i> were sown in pots and supplemented with NaCl at 15-day stage. Si and EBL treatments were given at 20- and 25-day stages, respectively.	The spray of Si and EBL alone or in combination significantly increased the growth and photosynthetic traits in the presence/absence of NaCl stress. A combined effect of Si and EBL counters the damaged caused by the salt stress.
Salicylic acid [99]	<i>B. napus</i>	10 days seedlings grown for 48 h in Hyponex solution with 100 and 200 mM NaCl and with and without spraying 100 µM salicylic acid	SA is an effective protectant in improving the activities of both antioxidant defense and glyoxalase enzymes in conferring salt stress tolerance in <i>B. napus</i> .

Table 3. Cont.

Aget	Plant	Experimental Design	Results
Salicylic acid [100]	<i>B. napus</i>	One-month-old plants, where four fully expanded leaves had appeared, were sprayed with salicylic acid (0, 0.5, 1 mM) on to the leaves. After the salicylic acid treatment, plants were grown under salt concentrations of (0, 4, 8 and 12) $\text{dsm}^{-1}$ for ten days. Plants were grown with 50 mM NaCl and were sprayed with 0.1, 0.5, and 1.0 mM salicylic acid (SA). Plants were harvested at 30 DAS (15 days after SA application)	SA application increased photosynthetic pigments (Chl a, b and carotenoids), protein and soluble sugars, free amino acids, including proline and MAD content compared to plants under salinity stress
Salicylic acid [101]	<i>B. juncea</i>	Twenty-day-old choysum plants were subjected to different salt stress levels (0, 100, 150 and 200 mM NaCl) by applying NaCl solutions were to the pots' soil. A week after the first salt stress was commenced; salicylic acid (1 mM) was foliar applied once a week until harvesting at 45 DAS.	The application of 0.5 mM SA alleviated the negative effects of 50 mM NaCl maximally, but 1.0 mM SA proved inhibitory. The effect of SA was more conspicuous in a salt-tolerant cultivar.
Salicylic acid [104]	<i>B. parachinensis</i>	Four-week-old cultivars were treated with NaCl (50, 100 and 150 mM) and SA were applied by spraying to the aerial plant parts four times (at one-week interval) starting from the fifth week after germination up to the eighth week. Sampling was done when plants were 9 weeks old.	SA application induced tolerance to salinity stress in choysum plants due to the synchronized increase in activities of enzymatic and non-enzymatic antioxidants, enhanced efficiency of AsA-GSH cycle and the MG detoxification systems.
Salicylic acid [103]	<i>B. carinata</i>	The leaves of 29-day-old plants grown in presence or absence of saline conditions ( $4.2 \text{ dsm}^{-1}$ ) were sprayed with distilled water, HBL and/or SA and plant responses were studied at 30 days after sowing (24 h after spray) and 45 days after sowing.	SA significantly reduced the salinity-caused effects on the overall performance of plants and their antioxidant systems in both the cultivars.
28 homobrassinolide (HBL) salicylic acid [102]	<i>B. juncea</i>	The salinization and MeJA application were simultaneously carried out when plants were 16 days old. Ethanol (0.04%, <i>v/v</i> ) was added to the solution prepared for the foliar applications as solvent.	HBL excelled in its effects at both sampling stages. Toxic effects generated by salinity stress were completely overcome by the combination of the two hormones (HBL and SA) at 45 DAS
methyl jasmonate [105]	<i>B. napus</i>	At 4 weeks of cultivation, hydroponically cultivated plants were sprayed with phenolic acids: salicylic (SA) and trans-ferulic acid (FA) (10, 50, and 100 $\mu\text{M}$ ) and the NaCl concentration in the hydroponic solution was gradually increased up to 150 mM NaCl. The photosynthetic parameters were measured 72 h after salt application and plants were harvested for analysis.	Exogenously applied MeJA counteracted the inhibitory effects of NaCl by increasing relative water content, soluble sugar content and photosynthesis rate.
Ferulic acid (FA) Salicylic acid (SA) [73]	<i>B. rapa</i> L. ssp. <i>pekinensis</i>	The leaves of plants grown in a pots under 200 mM NaCl were sprayed with 5 mL of ALA solution at a concentration of 0 or 30 $\text{mg}\cdot\text{L}^{-1}$ at 35 and 40 DAS. Plants were harvested 10 days after initially being treated with ALA.	SA and FA treatments, with a concentration of 10 $\mu\text{M}$ , had attenuated effects on salt-stressed plants, causing a decrease in proline and SA level, and indicating that the plants suffered less metabolic disturbance. FA resulted in a better ameliorative effect on salt stress compared to SA.
5-aminolevulinic acid (ALA) [106]	<i>B. napus</i> L.		ALA improved salt tolerance by promoting the accumulation of chlorophyll and heme resulting from the increase of intermediate levels in the tetrapyrrole biosynthetic pathway, along with enhancing the proline accumulation in <i>B. napus</i> .

**Table 4.** List of root treatments by beneficial molecules and their ameliorative effects on Brassica species under salinity stress.

Aget	Brassica	Experimental Design	Results
GR24, strigolactone [107]	<i>B. napus</i>	Four-week-old plants grown in half-strength Hoagland nutrient solution were treated with salinities (0, 100, and 200 mM NaCl) and with presence or absence of GE24.	Strigolactones GR24 improve plant growth, photosynthesis, and alleviate oxidative stress.
Poly( $\gamma$ -glutamic acid) ( $\gamma$ -PGA) [108]	<i>B. napus</i>	A salinity model was simulated by exposing the hydroponically grown roots of rape seedlings to 100 mM NaCl solution for 48, 96 and 144 h with or without the presence of 20 mg/L $\gamma$ -PGA.	$\gamma$ -PGA improved resistance to salt stress in by activating the proline synthesis pathway and promoting proline accumulation.
silicone [109]	<i>B. napus</i>	12 DAS plants, growing semi-hydroponic, SiO <sub>2</sub> (1 mM) and NaCl (100 and 200 mM) were added in the solution—alone or in combination. The first leaves were harvested and used for quantifying parameters after 48 h of treatment exposure.	Si improved plant tolerance to salinity stress through enhancement of both antioxidant defense and glyoxalase systems that led to reduced oxidative damage and methylglyoxal toxicity.
melatonin [110]	<i>B. napus</i>	Nine-day-old seedlings analysed after growing in solution with different NaCl and melatonin concentrations.	Melatonin improves the H <sub>2</sub> O <sub>2</sub> -scavenging capacity, alleviate osmotic stress by promoting the accumulation of osmoregulators, facilitate root development and improve the biomass
melatonin [111] ( $\mu$ M) (MT) and lipoic acid (LA)	<i>B. napus</i>	Seeds were sown in Petri dishes (HS) with 0 or 100 mM NaCl or melatonin ( $\mu$ M) (MT) or lipoic acid (LA) treatments (0.5 $\mu$ M) below and above the seeds. Seedlings grown until the shoot attained 5 to 6 cm height and root attained 7 to 8 cm.	The alteration of metabolic pathways, redox modulation, and ions homeostasis in plant tissues by the combined LA and MT application are helpful towards the adaptation in a saline environment.
Serotonin [112]	<i>B. napus</i>	Hydroponically grown seedlings with two leaves were treated with serotonin (0, 50, 100, 200, 300 $\mu$ mol/L) under 0.75% NaCl.	Serotonin improved the ability of ROS scavenging, osmotic pressure regulation and promoting growth, thus alleviating the salinity of seedlings
Thiourea (TU) [113]	<i>B. juncea</i> var. TPM1	Hydroponically grown plants were subject to salinity stress (150 mM NaCl) with and without the presence of 75 $\mu$ M TU. Analysis were performed 7-d post-treatment.	Thiourea (TU, a ROS scavenger) has delineates salt stress ameliorating action. The ameliorative potential of TU towards NaCl stress was related with its ability to decrease ROS accumulation in roots and increase Na <sup>+</sup> accumulation in shoots.

#### 4. Conclusions

Soil salinization is a global problem that can lead to significant losses in agricultural production. In this review, we summarized and reviewed recent data on several microorganisms and molecules whose application may be beneficial for mitigating salt stress in *Brassica* plants.

Several *Brassica* species are able to establish mutualistic relationships with their microbiome that could help them cope with stress caused by increasing salinity. However, further interdisciplinary research is needed to characterize this positive relationship and the potential to utilize beneficial and/or salt-tolerant microbes as a biotic resource that could minimize the damage to *Brassica* plants caused by induced salt stress.

The knowledge of the mechanisms involved in the stress response of *Brassica* plants provided the impetus for the research, which focused on the development of different application methods that induce a better and faster response to salt stress and consequently help to mitigate the negative effects of salt stress. The application of the beneficial molecules can be done through the seed, known as seed priming, or at advanced stages of development through foliar spraying or root application. Several molecules show promising activity, but all of these experiments were conducted in the laboratory/under controlled conditions and additional experiments under field are needed to determine optimal conditions of application. According to the data we summarized, the experiments are also limited to some *Brassica* plant species and research should extend to a wider range of different species/cultivars. Moreover, many of the available data show the beneficial effects of certain biomolecules and microorganisms on seedlings or young plants under stress conditions. Further research should evaluate their potential beneficial effects on plants at commercial or horticultural maturity.

**Author Contributions:** Conceptualization, I.P. and D.Š.; writing—original draft preparation, I.P., D.Š., E.K., B.S.-S.; writing—review and editing, D.Š.; I.P., E.K., B.S.-S.; supervision, D.Š.; project administration, D.Š., I.P., B.S.-S.; funding acquisition, I.P., B.S.-S., D.Š. All authors have read and agreed to the published version of the manuscript.

**Funding:** This article is the result of activities on the project “Exploring adaptation potential of rhizosphere microbiome to climate change: towards sustainable agriculture in the future- PERSPIRE” (KK.05.1.1.02.0001) funded by the European Regional Development Fund (ERDF). D.Š. research is supported by Grant for Scientific Research and Artistic Work from the University North under the no. UNIN-BIOTEH-21-1-1.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. Shah, A.N.; Tanveer, M.; Abbas, A.; Fahad, S.; Baloch, M.S.; Ahmad, M.I.; Saud, S.; Song, Y. Targeting salt stress coping mechanisms for stress tolerance in Brassica: A research perspective. *Plant. Physiol. Biochem.* **2021**, *158*, 53–64. [[CrossRef](#)]
2. FAO (Food and Agriculture Organization of the United Nations). *Global Map of Salt-Affected Soils*; FAO: Rome, Italy, 2021.
3. Shahid, S.A.; Zaman, M.; Heng, L. Soil Salinity: Historical Perspectives and a World Overview of the Problem. In *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*; Shahid, S.A., Zaman, M., Heng, L., Eds.; Springer Open: Basel, Switzerland, 2018; pp. 43–53.
4. Šamec, D.; Salopek-Sondi, B. Cruciferous (Brassicaceae) vegetables. In *Nonvitamin and Nonmineral Nutritional Supplements*; Nabavi, S.M., Sanches Silva, T., Eds.; Academic Press: Cambridge, MA, USA, 2019; pp. 195–202.
5. Ashraf, M. Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid Brassica species in relation to their diploid parents. *Environ. Exp. Bot.* **2001**, *45*, 155–163. [[CrossRef](#)]
6. Chakraborty, K.; Bose, J.; Shabala, L.; Eyles, A.; Shabala, S. Evaluating relative contribution of osmotolerance and tissue tolerance mechanisms toward salinity stress tolerance in three Brassica species. *Physiol. Plant.* **2016**, *158*, 135–151. [[CrossRef](#)] [[PubMed](#)]



7. Pavlović, I.; Mlinarić, S.; Tarkowská, D.; Oklestková, J.; Novák, O.; Lepeduš, H.; Vujčić Bok, V.; Radić Brkanac, S.; Strnad, M.; Salopek-Sondi, B. Early Brassica crops responses to salinity stress: A comparative analysis between Chinese cabbage, White cabbage and Kale. *Front. Plant. Sci.* **2019**, *10*, 450. [[CrossRef](#)] [[PubMed](#)]
8. Purty, R.S.; Kumar, G.; Singla-Pareek, S.L.; Pareek, A. Towards salinity tolerance in Brassica: An overview. *Physiol. Mol. Biol. Plants* **2018**, *14*, 39–49. [[CrossRef](#)]
9. Linić, I.; Šamec, D.; Grúz, J.; Vujčić Bok, V.; Strnad, M.; Salopek Sondi, B. Involvement of Phenolic Acids in Short-Term Adaptation to Salinity Stress is Species-Specific among Brassicaceae. *Plants* **2019**, *8*, 155. [[CrossRef](#)]
10. Šamec, D.; Linić, I.; Salopek-Sondi, B. Salinity Stress as an Elicitor for Phytochemicals and Minerals Accumulation in Selected Leafy Vegetables of Brassicaceae. *Agronomy* **2021**, *11*, 361. [[CrossRef](#)]
11. Westman, S.M.; Kloth, K.J.; Hanson, J.; Ohlsson, A.B.; Albrechtsen, B.R. Defence priming in Arabidopsis—A Meta-Analysis. *Sci. Rep.* **2019**, *9*, 13309. [[CrossRef](#)]
12. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant. Sci.* **2012**, *17*, 478–486. [[CrossRef](#)]
13. Mauchline, T.H.; Malone, J.G. Life in earth—The root microbiome to the rescue? *Curr. Opin. Microbiol.* **2017**, *37*, 23–28. [[CrossRef](#)]
14. Otlewska, A.; Migliore, M.; Dybka-Stepien, K.; Manfredini, A.; Struszczyk-Swita, K.; Napoli, R.; Białkowska, A.; Canfora, L.; Pinzari, F. When Salt Meddles Between Plant, Soil, and Microorganisms. *Front. Plant. Sci.* **2020**, *11*, 553087. [[CrossRef](#)] [[PubMed](#)]
15. De Zelicourt, A.; Al-Yousif, M.; Hirt, H. Rhizosphere microbes as essential partners for plant stress tolerance. *Mol. Plant.* **2013**, *6*, 242–245. [[CrossRef](#)] [[PubMed](#)]
16. Nadeem, S.M.; Ahmad, M.; Zahir, Z.A.; Javaid, A.; Ashraf, M. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* **2014**, *32*, 429–448. [[CrossRef](#)] [[PubMed](#)]
17. Acosta-Motos, J.R.; Ortuño, M.F.; Bernal-Vicente, A.; Diaz-Vivancos, P.; SanchezBlanco, M.J.; Hernandez, J.A. Plant responses to salt stress: Adaptive mechanisms. *Agronomy* **2017**, *7*, 18. [[CrossRef](#)]
18. Rodriguez, R.; Redman, R. More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *J. Exp. Bot.* **2008**, *59*, 1109–1114. [[CrossRef](#)]
19. Vaishnav, A.; Shukla, A.K.; Sharma, A.; Kumar, R.; Choudhary, D.K. Endophytic Bacteria in Plant Salt Stress Tolerance: Current and Future Prospects. *J. Plant. Growth Regul.* **2019**, *38*, 650–668. [[CrossRef](#)]
20. Yan, N.; Marschner, P.; Cao, W.H.; Zuo, C.Q.; Qin, W. Influence of salinity and water content on soil microorganisms. *Int. Soil Water Conserv. Res.* **2015**, *3*, 316–323. [[CrossRef](#)]
21. Kumar, A.; Singh, S.; Gaurav, A.K.; Srivastava, S.; Verma, J.P. Plant growthpromoting bacteria: Biological tools for the mitigation of salinity stress in plants. *Front. Microbiol.* **2020**, *11*, 1216. [[CrossRef](#)]
22. Abdul Rahman, N.S.N.; Abdul Hamid, N.W.; Nadarajah, K. Effects of Abiotic Stress on Soil Microbiome. *Int. J. Mol. Sci.* **2021**, *22*, 9036. [[CrossRef](#)]
23. Mokrani, S.; Nabti, E.; Cruz, C. Current Advances in Plant Growth Promoting Bacteria Alleviating Salt Stress for Sustainable Agriculture. *Appl. Sci.* **2020**, *10*, 7025. [[CrossRef](#)]
24. Andronov, E.E.; Petrova, S.N.; Pinaev, A.G.; Pershina, E.V.; Rakhimgaliyeva, S.; Akhmedenov, K.M.; Gorobets, A.V.; Sergaliev, N.K. Analysis of the structure of microbial community in soils with different degrees of salinization using T-RFLP and real-time PCR techniques. *Eurasian Soil Sci.* **2012**, *45*, 147–156. [[CrossRef](#)]
25. Egamberdieva, D.; Shurigin, V.; Gopalakrishnan, S.; Sharma, R. Growth and symbiotic performance of chickpea (*Cicer arietinum*) cultivars under saline soil conditions. *J. Biol. Chem. Res.* **2014**, *31*, 333–341.
26. Vaishnav, A.; Kumari, S.; Jain, S.; Varma, A.; Tuteja, N.; Choudhary, D.K. PGPR mediated expression of salt tolerance gene in soybean through volatiles under sodium nitroprusside. *J. Basic Microbiol.* **2016**, *56*, 1–15. [[CrossRef](#)]
27. Liu, H.; Carvalhais, L.C.; Crawford, M.; Singh, E.; Dennis, P.G.; Pieterse, C.M.J.; Schenk, P.M. Inner plant values: Diversity, colonization and benefits from endophytic bacteria. *Front. Microbiol.* **2017**, *8*, 2552. [[CrossRef](#)]
28. Das, P.; Behera, B.K.; Meena, D.K.; Azmi, S.A.; Chatterjee, S.; Meena, K.; Sharm, A.P. Salt stress tolerant genes in halophilic and halotolerant bacteria: Paradigm for salt stress adaptation and osmoprotection. *Int. J. Curr. Microbiol. App. Sci.* **2015**, *4*, 642–658.
29. Gopalakrishnan, S.; Sathya, A.; Vijayabharathi, R.; Varshney, R.K.; Gowda, C.L.; Krishnamurthy, L. Plant growth promoting rhizobia: Challenges and opportunities. *3 Biotech* **2015**, *5*, 355–377. [[CrossRef](#)]
30. Ferreira, M.J.; Silva, H.; Cunha, A. Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: A review. *Pedosphere* **2019**, *29*, 409–420. [[CrossRef](#)]
31. Sunita, K.; Mishra, I.; Mishra, J.; Prakash, J.; Arora, N.K. Secondary Metabolites From Halotolerant Plant Growth Promoting Rhizobacteria for Ameliorating Salinity Stress in Plants. *Front. Microbiol.* **2020**, *11*, 567768. [[CrossRef](#)]
32. Choudhary, D.K.; Kasotia, A.; Jain, S.; Vaishnav, A.; Kumari, S.; Sharma, K.P.; Varma, A. Bacterial mediated tolerance and resistance to plants under abiotic and biotic stresses. *J. Plant. Growth. Regul.* **2015**, *35*, 276–300. [[CrossRef](#)]
33. Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* **2015**, *79*, 293–320. [[CrossRef](#)]
34. Jalil, S.U.; Ansari, M.I. Plant microbiome and its functional mechanism in response to environmental stress. *Int. J. Green Pharm.* **2018**, *12*, S81.

35. Li, H.S.; Lei, P.; Pang, X.; Li, S.; Xu, H.; Xu, Z.Q.; Feng, X.H. Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. *Appl. Soil. Ecol.* **2017**, *119*, 26–34. [[CrossRef](#)]
36. Cheng, Z.; Woody, O.Z.; McConkey, B.J.; Glick, B.R. Combined effects of the plant growth-promoting bacterium *Pseudomonas putida* UW4 and salinity stress on the *Brassica napus* proteome. *Appl. Soil. Ecol.* **2012**, *61*, 255–263. [[CrossRef](#)]
37. Abdel Latef, A.A.H.; Omer, A.M.; Badawy, A.A.; Osman, M.S.; Ragaey, M.M. Strategy of Salt Tolerance and Interactive Impact of *Azotobacter chroococcum* and/or *Alcaligenes faecalis* Inoculation on Canola (*Brassica napus* L.) Plants Grown in Saline Soil. *Plants* **2021**, *10*, 110. [[CrossRef](#)] [[PubMed](#)]
38. Stassinou, P.M.; Rossi, M.; Borromeo, I.; Capo, C.; Beninati, S.; Forni, C. Amelioration of salt stress tolerance in rapeseed (*Brassica napus*) cultivars by seed inoculation with *Arthrobacter globiformis*. *Plant. Biosyst.* **2021**, in press. [[CrossRef](#)]
39. Banaei-Asl, F.; Bandehagh, A.; Uliaei, E.D.; Farajzadeh, D.; Sakata, K.; Mustafa, G.; Komatsu, S. Proteomic analysis of canola root inoculated with bacteria under salt stress. *J. Proteom.* **2015**, *124*, 88–111. [[CrossRef](#)]
40. Ahmad, P.; Hashem, A.; Abd-Allah, E.F.; Alqarawi, A.A.; John, R.; Egamberdieva, D.; Gucel, S. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. *Front. Plant. Sci.* **2015**, *6*, 868. [[CrossRef](#)]
41. Phour, M.; Sindhu, S.S. Amelioration of salinity stress and growth stimulation of mustard (*Brassica juncea* L.) by salt-tolerant *Pseudomonas* species. *Appl. Soil. Ecol.* **2020**, *149*, 103518. [[CrossRef](#)]
42. Hong, B.H.; Joe, M.M.; Selvakumar, G.; Kim, K.Y.; Choi, J.H.; Sa, T.M. Influence of salinity variations on exocellular polysaccharide production, biofilm formation and flocculation in halotolerant bacteria. *J. Environ. Biol.* **2017**, *38*, 657–664. [[CrossRef](#)]
43. Woo, O.-G.; Kim, H.; Kim, J.-S.; Keum, H.L.; Lee, K.-C.; Sul, W.J.; Lee, J.-H. *Bacillus subtilis* strain GOT9 confers enhanced tolerance to drought and salt stresses in *Arabidopsis thaliana* and *Brassica campestris*. *Plant. Physiol. Biochem.* **2020**, *148*, 359–367. [[CrossRef](#)]
44. Lee, G.W.; Lee, K.-J.; Chae, J.-C. *Herbaspirillum* sp. strain GW103 alleviates salt stress in *Brassica rapa* L. ssp. *pekinensis*. *Protoplasma* **2015**, *253*, 655–661. [[CrossRef](#)] [[PubMed](#)]
45. Mahmood, A.; Can Turgay, O.; Farooq, M.; Hayat, R. Seed biopriming with plant growth promoting rhizobacteria: A review. *FEMS Microbiol Ecol.* **2016**, *92*, 1–14. [[CrossRef](#)]
46. Liang, W.; Ma, X.; Wan, P.; Liu, L. Plant salt-tolerance mechanism: A review. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 286–291. [[CrossRef](#)] [[PubMed](#)]
47. Patel, M.K.; Kumar, M.; Li, W.; Luo, Y.; Burrett, D.J.; Alkan, N.; Tran, L.-S.P. Enhancing salt tolerance of plants: From metabolic reprogramming to exogenous chemical treatments and molecular approaches. *Cells* **2020**, *9*, 2492. [[CrossRef](#)] [[PubMed](#)]
48. Wani, S.H.; Kumar, V.; Khare, T.; Guddimalli, R.; Parveda, M.; Solymosi, K.; Suprasanna, P.; Kishor, P.B.K. Engineering salinity tolerance in plants: Progress and prospects. *Planta* **2020**, *251*, 76. [[CrossRef](#)]
49. Hayat, S.; Hayat, Q.; Alyemini, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of proline under changing environments. *Plant. Signal. Behav.* **2012**, *7*, 1456–1466. [[CrossRef](#)]
50. Hayat, S.; Mir, B.A.; Wani, A.S.; Hasan, S.A.; Irfan, M.; Ahmad, A. Screening of salt-tolerant genotypes of *Brassica juncea* based on photosynthetic attributes. *J. Plant. Interact.* **2011**, *6*, 53–60. [[CrossRef](#)]
51. Mohamed, I.A.A.; Shalby, N.; Bai, C.; Qin, M.; Agami, R.A.; Jie, K.; Wang, B.; Zhou, G. Stomatal and photosynthetic traits are associated with investigating sodium chloride tolerance of *Brassica napus* L. cultivars. *Plants* **2020**, *9*, 62. [[CrossRef](#)]
52. Houmani, H.; Corpas, F.J. Differential responses to salt-induced oxidative stress in three phylogenetically related plant species: *Arabidopsis thaliana* (glycophyte), *Thellungiella salsuginea* and *Cakile maritima* (halophytes). Involvement of ROS and NO in the control of K<sup>+</sup>/Na<sup>+</sup> homeostasis. *AIMS Biophysics* **2016**, *3*, 380–397.
53. Assaha, D.V.M.; Ueda, A.; Saneoka, H.; Al-Yahyai, R.; Yaish, M.W. The Role of Na<sup>+</sup> and K<sup>+</sup> transporters in salt stress adaptation in glycophytes. *Front. Physiol.* **2017**, *8*, 509. [[CrossRef](#)]
54. Devireddy, A.R.; Zandalinas, S.I.; Fichman, Y.; Mittler, R. Integration of reactive oxygen species and hormone signaling during abiotic stress. *Plant. J.* **2021**, *105*, 459–476. [[CrossRef](#)]
55. Belmecheri-Cherifi, H.; Albacete, A.; Martínez-Andújar, C.; Pérez-Alfocea, F.; Abrous-Belbachir, O. The growth impairment of salinized fenugreek (*Trigonella foenum-graecum* L.) plants is associated to changes in the hormonal balance. *J. Plant. Physiol.* **2019**, *232*, 311–319. [[CrossRef](#)]
56. Hasanuzzaman, M.; Raihan, M.R.H.; Masud, A.A.C.; Rahman, K.; Nowroz, F.; Rahman, M.; Nahar, K.; Fujita, M. Regulation of Reactive Oxygen Species and Antioxidant Defense in Plants under Salinity. *Int. J. Mol. Sci.* **2021**, *22*, 9326. [[CrossRef](#)] [[PubMed](#)]
57. Yu, Z.; Duan, X.; Luo, L.; Dai, S.; Ding, Z.; Xia, G. How plant hormones mediate salt stress responses. *Trends Plant. Sci.* **2020**, *25*, 1117–1130. [[CrossRef](#)] [[PubMed](#)]
58. Smolko, A.; Bauer, N.; Pavlović, I.; Pěnčík, A.; Novák, O.; Salopek-Sondi, B. Altered root growth, auxin metabolism and distribution in *Arabidopsis thaliana* exposed to salt and osmotic stress. *Int. J. Mol. Sci.* **2021**, *22*, 7993. [[CrossRef](#)] [[PubMed](#)]
59. Galvan-Ampudia, C.S.; Testerink, C. Salt stress signals shape the plant root. *Curr. Opin. Plant. Biol.* **2011**, *14*, 296–302. [[CrossRef](#)]
60. Lu, C.; Chen, M.-X.; Liu, R.; Zhang, L.; Hou, X.; Liu, S.; Ding, X.; Jiang, Y.; Xu, J.; Zhang, J.; et al. Abscisic acid regulates auxin distribution to mediate maize lateral root development under salt stress. *Front. Plant. Sci.* **2019**, *10*, 716. [[CrossRef](#)] [[PubMed](#)]
61. Pavlović, I.; Pěnčík, A.; Novák, O.; Vujčić, V.; Radić Brkanac, S.; Lepeduš, H.; Strnad, M.; Salopek-Sondi, B. Short-term salinity stress response of *Brassica rapa* caused changes in auxin metabolism. *Plant. Physiol. Biochem.* **2018**, *125*, 74–84. [[CrossRef](#)]

62. Khan, M.A.; Hamayun, M.; Asaf, S.; Khan, M.; Yun, B.-W.; Kang, S.-M.; Lee, I.-J. Rhizospheric *Bacillus* spp. rescues plant growth under salinity stress via regulating gene expression, endogenous hormones, and antioxidant system of *Oryza sativa* L. *Front. Plant. Sci.* **2021**, *12*, 665590. [[CrossRef](#)]
63. Yu, Y.; Li, Y.; Yan, Z.; Duan, X. The role of cytokinins in plant under salt stress. *J. Plant. Growth Regul.* **2021**, *in press*. [[CrossRef](#)]
64. Bhanbhro, N.; Xiao, B.; Han, L.; Lu, H.; Wang, H.; Yang, C. Adaptive strategy of allohexaploid wheat to long-term salinity stress. *BMC Plant. Biol.* **2020**, *20*, 210. [[CrossRef](#)] [[PubMed](#)]
65. Anwar, A.; Liu, Y.; Dong, R.; Bai, L.; Yu, X.; Yansu Li, Y. The physiological and molecular mechanism of brassinosteroid in response to stress: A review. *Biol. Res.* **2018**, *51*, 46. [[CrossRef](#)] [[PubMed](#)]
66. Mostofa, M.G.; Li, W.; Nguyen, K.H.; Fujita, M.; Phan Tran, L.-S. Strigolactones in plant adaptation to abiotic stresses: An emerging avenue of plant research. *Plant. Cell Environ.* **2018**, *41*, 2227–2243. [[CrossRef](#)] [[PubMed](#)]
67. Riemann, M.; Dhakarey, R.; Hazman, M.; Miro, B.; Kohli, A.; Nick, P. Exploring Jasmonates in the Hormonal Network of Drought and Salinity Responses. *Front. Plant. Sci.* **2015**, *6*, 1077. [[CrossRef](#)] [[PubMed](#)]
68. Farhangi-Abri, S.; Ghassemi-Golezani, K. Jasmonates: Mechanisms and functions in abiotic stress tolerance of plants. *Biocatal. Agric. Biotechnol.* **2019**, *20*, 101210. [[CrossRef](#)]
69. Khan, M.I.R.; Fatma, M.; Per, T.S.; Anjum, N.A.; Khan, N.A. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant. Sci.* **2015**, *6*, 462. [[CrossRef](#)]
70. Miura, K.; Tada, Y. Regulation of water, salinity, and cold stress responses by salicylic acid. *Front. Plant. Sci.* **2014**, *5*, 4. [[CrossRef](#)]
71. Ma, X.; Zheng, J.; Zhang, X.; Hu, Q.; Qian, R. Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyllaceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. *Front. Plant. Sci.* **2017**, *8*, 600. [[CrossRef](#)]
72. Naeem, M.; Basit, A.; Ahmad, I.; Mohamed, H.I.; Wasila, H. Effect of Salicylic Acid and Salinity Stress on the Performance of Tomato Plants. *Gesunde Pflanzen* **2020**, *72*, 393–402. [[CrossRef](#)]
73. Linić, I.; Mlinarić, S.; Brkljačić, L.; Pavlović, I.; Smolko, A.; Salopek-Sondi, B. Ferulic acid and Salicylic acid foliar treatments reduce short-term salt stress in Chinese cabbage by increasing phenolic compounds accumulation and photosynthetic performance. *Plants* **2021**, *10*, 2346. [[CrossRef](#)]
74. Riyazuddin, R.; Verma, R.; Singh, K.; Nisha, N.; Keisham, M.; Bhati, K.K.; Kim, S.T.; Gupta, R. Ethylene: A master regulator of salinity stress tolerance in plants. *Biomolecules* **2020**, *10*, 959. [[CrossRef](#)] [[PubMed](#)]
75. Šamec, D.; Karalija, E.; Šola, I.; Vujčić Bok, V.; Salopek-Sondi, B. The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants* **2021**, *10*, 118. [[CrossRef](#)] [[PubMed](#)]
76. Buhmann, A.; Papenbrock, J. An economic point of view of secondary compounds in halophytes. *Funct. Plant. Biol.* **2013**, *40*, 952–967. [[CrossRef](#)] [[PubMed](#)]
77. Lee, S.S.; Kim, J.H. Total sugars,  $\alpha$ -amylase activity, and emergence after priming of normal and aged rice seeds. *Korean J. Crop. Sci.* **2000**, *45*, 108–111.
78. Hussain, S.; Zheng, M.; Khan, F.; Khaliq, A.; Fahad, S.; Peng, S.; Huang, J.; Cui, K.; Nie, L. Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. *Sci. Rep.* **2015**, *5*, 8101. [[CrossRef](#)]
79. Brocklehurst, P.A.; Dearman, J. Interaction between seed priming treatments and nine seed lots of carrot, celery and onion II. Seedling emergence and plant growth. *Ann. Appl. Biol.* **2008**, *102*, 583–593. [[CrossRef](#)]
80. Karalija, E.; Selović, A. The effect of hydro and proline seed priming on growth, proline and sugar content, and antioxidant activity of maize under cadmium stress. *Environ. Sci. Pollut. Res.* **2018**, *25*, 33370–33380. [[CrossRef](#)]
81. Mladenov, V.; Fotopoulos, V.; Kaiserli, E.; Karalija, E.; Maury, S.; Baranek, M.; Segal, N.; Testillano, P.S.; Vassileva, V.; Pinto, G.; et al. Deciphering the Epigenetic Alphabet Involved in Transgenerational Stress Memory in Crops. *Int. J. Mol. Sci.* **2021**, *22*, 7118. [[CrossRef](#)]
82. Karalija, E.; Selović, A.; Dahija, S.; Demir, A.; Samardžić, J.; Vrobel, O.; Čavar-Zeljko, S.; Parić, A. Use of seed priming to improve Cd accumulation and tolerance in *Silene sendtneri*, novel Cd hyper-accumulator. *Ecotoxicol. Environ. Saf.* **2021**, *210*, 111882. [[CrossRef](#)]
83. Nawaz, A.; Amjad, M.; Pervez, M.A.; Afzal, I. Effect of halopriming on germination and seedling vigor of tomato. *Afr. J. Agric. Res.* **2011**, *6*, 3551–3559.
84. Farhoudi, R.; Sharifzadeh, F.; Poustini, K.; Makkizadeh, M.T.; Kochak Por, M. The effects of NaCl priming on salt tolerance in canola (*Brassica napus*) seedlings grown under saline conditions. *Seed Sci. Technol.* **2007**, *35*, 754–759. [[CrossRef](#)]
85. Hassini, I.; Baenas, N.; Moreno, D.A.; Carvajal, M.; Boughanmi, N.; Martinez Ballesta, M.D.C. Effects of seed priming, salinity and methyl jasmonate treatment on bioactive composition of *Brassica oleracea* var. capitata (white and red varieties) sprouts. *J. Sci. Food Agric.* **2017**, *97*, 2291–2299. [[CrossRef](#)] [[PubMed](#)]
86. Abdollahi, F.A.R.Z.I.N.; Jafari, L.E.I.L.A. Effect of NaCl and KNO<sub>3</sub> priming on seed germination of canola (*Brassica napus* L.) under salinity conditions. *Int. J. Agric.* **2012**, *2*, 573–579.
87. Begum, N.; Gul, H.; Hamayun, M.; Rahman, I.U.; Ijaz, F.; Sohail, Z.I.; Afzal, A.; Afzal, M.; Ullah, A.; Karim, S. Influence of seed priming with ZnSO<sub>4</sub> and CuSO<sub>4</sub> on germination. *Middle East. J. Sci. Res.* **2014**, *22*, 879–885.
88. Kubala, S.; Wojtyła, Ł.; Quinet, M.; Lechowska, K.; Lutts, S.; Garnczarska, M. Enhanced expression of the proline synthesis gene P5CSA in relation to seed osmopriming improvement of *Brassica napus* germination under salinity stress. *J. Plant. Physiol.* **2015**, *183*, 1–12. [[CrossRef](#)] [[PubMed](#)]

89. Hassanpouraghdam, M.B.; Pardaz, J.E.; Akhtar, N.F. The effect of osmo-priming on germination and seedling growth of *Brassica napus* L. under salinity conditions. *J. Food Agric. Environ.* **2009**, *7*, 620–622.
90. Jamil, M.; Ashraf, M.; Rha, E. Alleviation of salt stress using gibberellic acid in Chinese cabbage. *Acta Agron. Hung.* **2012**, *60*, 345–355. [[CrossRef](#)]
91. Benincasa, P.; Pace, R.; Quinet, M.; Lutts, S. Effect of salinity and priming on seedling growth in rapeseed (*Brassica napus* var *oleifera* Del.). *Acta Sci. Agron.* **2013**, *35*, 479–486. [[CrossRef](#)]
92. Akram, N.A.; Hafeez, N.; Farid-ul-Haq, M.; Ahmad, A.; Sadiq, M.; Ashraf, M. Foliage application and seed priming with nitric oxide causes mitigation of salinity-induced metabolic adversaries in broccoli (*Brassica oleracea* L.) plants. *Acta Physiol. Plant.* **2020**, *42*, 1–9. [[CrossRef](#)]
93. Shahbaz, M.; Noreen, N.; Perveen, S. Triacontanol modulates photosynthesis and osmoprotectants in canola (*Brassica napus* L.) under saline stress. *J. Plant. Interact.* **2013**, *8*, 350–359. [[CrossRef](#)]
94. Yan, M. Hydro-priming increases seed germination and early seedling growth in two cultivars of Napa cabbage (*Brassica rapa* subsp. *pekinensis*) grown under salt stress. *J. Hortic. Sci. Biotechnol.* **2016**, *91*, 421–426. [[CrossRef](#)]
95. El-Badri, A.M.; Batoool, M.; Wang, C.; Hashem, A.M.; Tabl, K.M.; Nishawy, E.; Kuai, J.; Zhoua, G.; Wang, B. Selenium and zinc oxide nanoparticles modulate the molecular and morpho-physiological processes during seed germination of *Brassica napus* under salt stress. *Ecotoxicol. Environ. Saf.* **2021**, *225*, 112695. [[CrossRef](#)] [[PubMed](#)]
96. Wani, A.S.; Ahmad, A.; Hayat, S.; Tahir, I. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea* cultivars? *Environ. Sci. Pollut. Res.* **2016**, *23*, 13413–13423. [[CrossRef](#)] [[PubMed](#)]
97. Wani, A.S.; Ahmad, A.; Hayat, S.; Tahir, I. Epibrassinolide and proline alleviate the photosynthetic and yield inhibition under salt stress by acting on antioxidant system in mustard. *Plant. Physiol. Biochem.* **2019**, *135*, 385–394. [[CrossRef](#)]
98. Siddiqui, H.; Yusuf, M.; Faraz, A.; Faizan, M.; Sami, F.; Hayat, S. 24-Epibrassinolide supplemented with silicon enhances the photosynthetic efficiency of *Brassica juncea* under salt stress. *S. Afr. J. Bot.* **2018**, *118*, 120–128. [[CrossRef](#)]
99. Hasanuzzaman, M.; Alam, M.; Nahar, K.; Al Mahmud, J.; Ahmed, K.U.; Fujita, M.; Masayuki, F. Exogenous salicylic acid alleviates salt stress-induced oxidative damage in *Brassica napus* by enhancing the antioxidant defense and glyoxalase systems. *Aust. J. Crop. Sci.* **2014**, *8*, 631–639.
100. Baghizadeh, A.; Salarizadeh, M.R.; Abaasi, F. Effects of Salicylic acid on some physiological and biochemical parameters of *Brassica napus* L. (Canola) under salt stress. *Int. J. Agric. Sci.* **2014**, *4*, 147–152.
101. Syeed, S.; Anjum, N.A.; Nazar, R.; Iqbal, N.; Masood, A.; Khan, N.A. Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. *Acta Physiol. Plant.* **2011**, *33*, 877–886.
102. Hayat, S.; Maheshwari, P.; Wani, A.S.; Irfan, M.; Alyemeni, M.N.; Ahmad, A. Comparative effect of 28 homobrassinolide and salicylic acid in the amelioration of NaCl stress in *Brassica juncea* L. *Plant. Physiol. Biochem.* **2012**, *53*, 61–68. [[CrossRef](#)]
103. Husen, A.; Iqbal, M.; Sohrab, S.S.; Ansari, M.K.A. Salicylic acid alleviates salinity-caused damage to foliar functions, plant growth and antioxidant system in Ethiopian mustard (*Brassica carinata* A. Br.). *Agric. Food Secur.* **2018**, *7*, 44. [[CrossRef](#)]
104. Kamran, M.; Xie, K.; Sun, J.; Wang, D.; Shi, C.; Lu, Y.; Gu, W.; Xu, P. Modulation of growth performance and coordinated induction of ascorbate-glutathione and methylglyoxal detoxification systems by salicylic acid mitigates salt toxicity in choysum (*Brassica parachinensis* L.). *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109877. [[CrossRef](#)] [[PubMed](#)]
105. Ahmadi, F.I.; Karimi, K.; Struik, P.C. Effect of exogenous application of methyl jasmonate on physiological and biochemical characteristics of *Brassica napus* L. cv. Talaye under salinity stress. *S. Afr. J. Bot.* **2018**, *115*, 5–11. [[CrossRef](#)]
106. Xiong, J.-L.; Wang, H.-C.; Tan, X.-Y.; Zhang, C.-L.; Naeem, M.S. 5-aminolevulinic acid improves salt tolerance mediated by regulation of tetrapyrrole and proline metabolism in *Brassica napus* L. seedlings under NaCl stress. *Plant. Physiol. Biochem.* **2018**, *124*, 88–99. [[CrossRef](#)]
107. Ma, N.; Hu, C.; Wan, L.; Hu, Q.; Xiong, J.; Zhang, C. Strigolactones Improve Plant Growth, Photosynthesis, and Alleviate Oxidative Stress under Salinity in Rapeseed (*Brassica napus* L.) by Regulating Gene Expression. *Front. Plant. Sci.* **2017**, *8*, 1671. [[CrossRef](#)] [[PubMed](#)]
108. Lei, P.; Xu, Z.; Liang, J.; Luo, X.; Zhang, Y.; Feng, X.; Xu, H. Poly( $\gamma$ -glutamic acid) enhanced tolerance to salt stress by promoting proline accumulation in *Brassica napus* L. *Plant. Growth Regul.* **2015**, *78*, 233–241. [[CrossRef](#)]
109. Hasanuzzaman, M.; Nahar, K.; Rohman, M.M.; Anee, T.I.; Huang, Y.; Fujita, M. Exogenous Silicon Protects *Brassica napus* Plants from Salinity-Induced Oxidative Stress Through the Modulation of AsA-GSH Pathway, Thiol-Dependent Antioxidant Enzymes and Glyoxalase Systems. *Gesunde Pflanz.* **2018**, *70*, 185–194. [[CrossRef](#)]
110. Liu, Z.; Jun-song, C.; Jing-jing, L.; Guang-yuan, L.; Chun-sheng, L.; Gui-ping, F.; Xue-kun, Z.; Hai-qing, M.; Qing-yun, L.; ZOU Xi-ling, Z.; et al. Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings. *J. Integr. Agric.* **2018**, *17*, 328–335.
111. Javeed, H.M.R.; Ali, M.; Skalicky, M.; Nawaz, F.; Qamar, R.; Rehman, A.u.; Faheem, M.; Mubeen, M.; Iqbal, M.M.; Rahman, M.H.u.; et al. Lipoic Acid Combined with Melatonin Mitigates Oxidative Stress and Promotes Root Formation and Growth in Salt-Stressed Canola Seedlings (*Brassica napus* L.). *Molecules* **2021**, *26*, 3147. [[CrossRef](#)]
112. Liu, Y.; Ding, X.; Lv, Y.; Cheng, Y.; Li, C.; Yan, L.; Tian, S.; Zou, X. Exogenous Serotonin Improves Salt Tolerance in Rapeseed (*Brassica napus* L.) Seedlings. *Agronomy* **2021**, *11*, 400. [[CrossRef](#)]
113. Srivastava, A.K.; Sablok, G.; Hackenberg, M.; Deshpande, U.; Suprasanna, P. Thiourea priming enhances salt tolerance through co-ordinated regulation of microRNAs and hormones in *Brassica juncea*. *Sci Rep.* **2017**, *7*, 45490. [[CrossRef](#)]