

## Review Article

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# Nanobiotechnology and microbial influence on cold adaptation in plants

<https://doi.org/10.1515/ntrev-2024-0059>

received December 24, 2023; accepted June 24, 2024

**Abstract:** Cold stress has an immediate impact on plant structure and function. A large number of free radicals cause oxidative stress in plants. Cold stress causes altered membrane permeability, lipid peroxidation, and DNA damage. It denatures enzymes and disrupts plant metabolism. Different methods are being investigated for acclimatizing plants subjected to cold stress. Nanobiotechnology and bacterial strains are growing agricultural strategies. Nanoparticles' (NPs) unique qualities (small size, high mobility, biocompatibility, low cost, and increased reactivity) make them ideal candidates in agriculture. NP and bacterial applications

maintain plastid structure and function, enhance antioxidant activities, secondary metabolites, and hormone expression, and reduce electrolyte leakage. They increase the number and content of proteins involved in oxidation-reduction reactions, hormone pathways, stress signaling, and reactive oxygen species detoxification under cold stress conditions. Chitosan, zinc oxide, and titanium dioxide NPs can help plants with cold stress. Meanwhile, bacterial strains in the genus *Bacillus* and *Pseudomonas* have been tested for cold tolerance. These strategies also upregulate antifreeze proteins, which are essential for the storage of plant products. Nano-bio-fertilizers should be prepared for the sustainable development of plants under low temperatures.

**Keywords:** abiotic stress, plant physiology, gene regulation, nanobiotechnology, cold stress, chilling stress, plant growth-promoting rhizobacteria

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## 1 Introduction

### 1.1 Cold stress vs plants

Most crops face abiotic stresses at different developmental stages that both quantitatively and qualitatively affect their production. The growing population demands more food production. The current population is 8 billion, which will be 9.7 billion in 2050, so production should be increased to avoid hunger and food scarcity. The agricultural lands are facing stressful conditions. The abiotic stresses, on average, cause more than 60% loss in crop production. They retard seed germination, crop establishment, and damage the structure and function of cells. The temperature fluctuation is uncertain, which causes severe threats to crops. Crop's sensitivity towards temperature depends on various factors like type of species, developmental stage, duration, and severity of stress [1,2]. The occurrence of abiotic stress results in altered metabolism and stunted growth. They also upregulate the defensive genes to combat the adverse effects of external stresses. Often, plants in the field face more than one stress

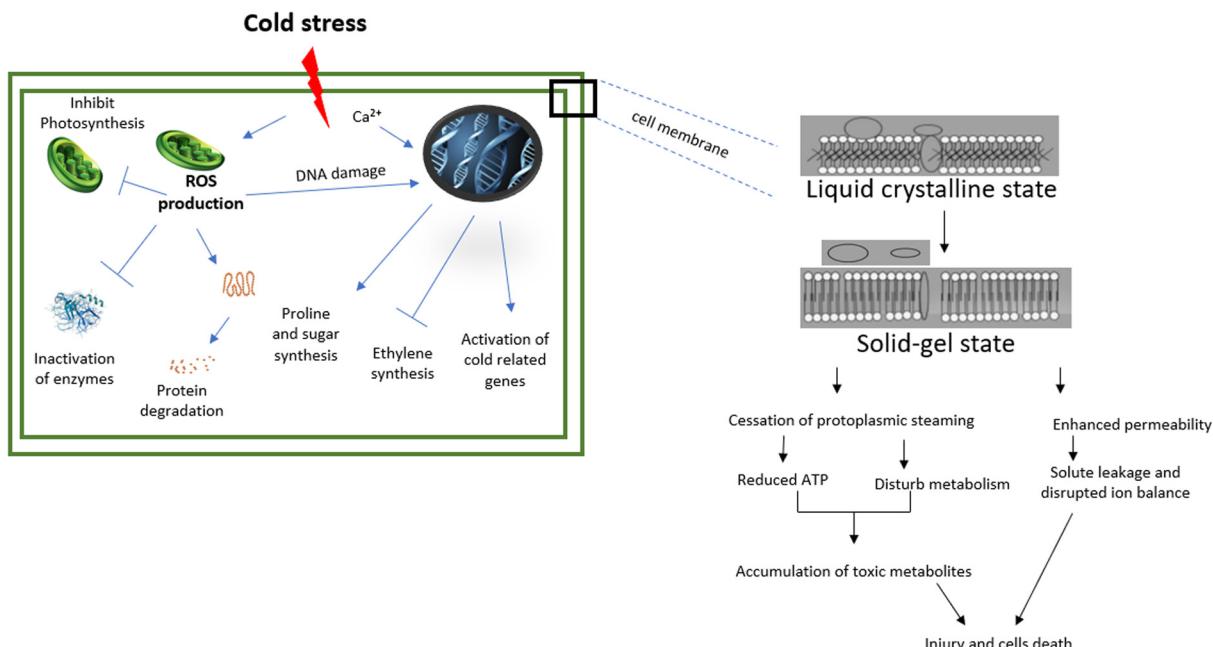
at a time, worsening the situation. For example, salinity also restricts water absorption from the soil. It causes water deficiency that affects the rate of gaseous exchange and rate of photosynthesis and also results in the deterioration of cellular components. The reduced water availability in salinity, drought, and cold stress retard enzymatic activities which results in the inhibition of plant development [3,4]. Cold stress undermines plant metabolism and causes morphological, physiological, and biochemical changes that affect plant life. Acute temperature changes cause membrane damage, chlorosis, and necrosis, influencing enzyme activity and cytoplasmic viscosity. Cold stress is a limiting factor that causes significant losses in agriculture because many crops, particularly vegetables, are sensitive to cold temperatures. They alter membrane fluidity, which reduces transport and enzyme activity and causes crystal formation that ruptures cell structures [5]. Plant reproductive stages are more vulnerable to cold stress, which causes pollen sterility, flower abscission, deformed pollen tube germination, and ovule abortion, that results in less production. The onset of cold stress at the grain-filling stage reduces crop yield. Cold stress disrupts meiosis, degrades anther protein, pollen tube deformation, stunted development of pollen grains, reduction in the size of style, reduced stigma receptivity, damage to embryo sac components, and arrest of fertilization in male and female gametophytes [6].

In response to cold, the leaf and flower bud halt physiological processes and remain dormant. Dormancy protects these buds against cold weather by allowing them to adapt

to freezing temperatures. Carbohydrates and other energy-yielding metabolites accumulate in the buds and maintain osmotic potential. They also act as energy reserves. The genes control bud dormancy, avoid their opening in harsh spell. When the buds have enough freezing, and the temperature begins to rise, they will grow. If the buds do not get enough chilling in cold temperatures, it results in more buttoning, less fruit set, and delayed foliation, lowering fruit quality [7]. Cold stress causes altered membrane permeability, lipid peroxidation, and DNA damage. It denatures enzymes and generally disrupts plant metabolism (Figure 1) [8].

Low temperatures produce several physiological issues in chilling sensitive fruits, such as tissue browning, a woolly or dry texture, and reduced membrane permeability, which causes irregularities in cell metabolism. When plants are exposed to cold temperatures, there is cross-talk at the molecular and cellular levels between ripening and senescence processes. It has been observed that the bZIP protein is responsive to cold stress, with *Ppbzip5*, *Ppbzip1*, *Ppbzip13*, *Ppbzip2*, *Ppbzip11*, *Ppbzip12*, and *Ppbzip7* showing differential expression in response to chilling stress. Transcription factors such as DREBs and CBF regulate abscisic acid pathways. *TaCBF15* and *TaCBF14* are engaged in cold signaling in transgenic plants (*Triticum aestivum*), which improves plant cold tolerance [4,8].

Nanotechnology offers optimistic solutions for enhancing plant productivity under abiotic stress. It improves plant tolerance under stress conditions that generate



**Figure 1:** Effect of cold stress on plant cells.

economic benefits for farmers by enhancing crop productivity. It improves nutrient uptake by solubilizing and mobilizing them in the rhizosphere. Nanoparticles (NPs) support the growth of beneficial bacteria in the vicinity of roots. These microbes synthesize antioxidant enzymes that scavenge reactive oxygen species (ROS) and reduce the damaging effects of free radicals. In this way, they preserve the functional and structural integrity of plants. They improve plants' water use efficiency, and increase the chances of plant survival under a limited water supply. The development of nanobiotechnology can create a path for producing stress-tolerant high-yielding crops [9,10]. In the current era, microorganisms, including bacteria, are the cost-effective and non-toxic solution for reducing the use of chemicals in the fields. The combined use of bacteria and NPs improves the growth of plants. NPs improve the colony numbers in the rhizosphere and rhizoplane, while plant growth-promoting bacteria enhance plant development by direct and indirect mechanisms. The co-application of NPs and bacterial strains improves membrane damage and rate of photosynthesis and maintains the structural integrity of organelles [11,12].

This review bridges the knowledge gap between the application of nanobiotechnology and plant-beneficial bacterial strains on plants subjected to low-temperature stress. Plants receiving these treatments show the activation of enzymes and genes following similar pathways. This script acts as a pillar for developing useful products using nanobiotechnology and bacterial strains to make plants cold-tolerant.

## 1.2 Nanobiotechnology and PGPR vs plants

Nanobiotechnology is a cutting-edge technique for agricultural development that promotes long-term growth. It is a convergence of nanotechnology and biotechnology that encompasses diverse approaches from biology, physics, engineering, and chemistry. Nanobiotechnology has applications in every scientific subject. It has the potential to benefit crop development by improving nitrogen metabolism, carbohydrate, protein, and chlorophyll content, ascorbate peroxidase, superoxide dismutase, and catalase activities in leaves and also regulates gene expression. It increases the number and quantity of proteins involved in oxidation-reduction reactions, hormonal pathways, stress signaling, and ROS detoxification under stressful circumstances [13,14]. NPs improve plants' ability to adapt to stressful situations by improving hydraulic conductance, nutrient uptake, and transport. Their high surface-to-volume

ratio enhances their reactivity. Quantum dots, metal and metal oxide NPs, nanotubes, fullerenes, dendrimers, and other NPs are examples of NPs. They have irregular, spherical, and tubular morphologies and can be found in fused, agglomerated, aggregated, or solitary state [1,15]. The application of NPs addresses crop challenges such as climate change and biotic influences. They enhance soil characteristics, plant germination, and vegetative development (Figure 2). According to the literature, NPs also mitigate the adverse effects of heavy metal stress on plants [16].

NPs are used to minimize the effects of biotic and abiotic stressors on plants. They function as elicitors for secondary metabolite formation, modulate hormone expression, and activate defense-related genes. They stimulate the rubisco enzyme and enhance its carboxylation, that improves the rate of photosynthesis. They also increase the expression of the thylakoid membrane light-harvesting complex, tonoplast intrinsic proteins, essential intrinsic proteins, and plasma membrane intrinsic proteins. They protect proteins from denaturing and control their active forms inside the cells. They regulate proteins' shape, size, and surface charge, which play an essential role in protein activity. They also modify surfaces by conjugating proteins with other proteins and carbohydrates. They control the expression of microRNAs involved in various morphological, physiological, and metabolic processes [17].

Plant growth promoting rhizobacteria (PGPR) are soil bacteria active in colonizing plant roots, thriving in the rhizosphere, and nurturing plant development. This significant component of the soil microbiome, which comprises a wide range of bacteria, is known for its capacity to produce and release a range of regulatory substances close to plant roots, thus promoting plant development through their contributions to enhanced nutrient absorption, protection against phytopathogenic organisms, and reinforcement against a variety of environmental stressors. They

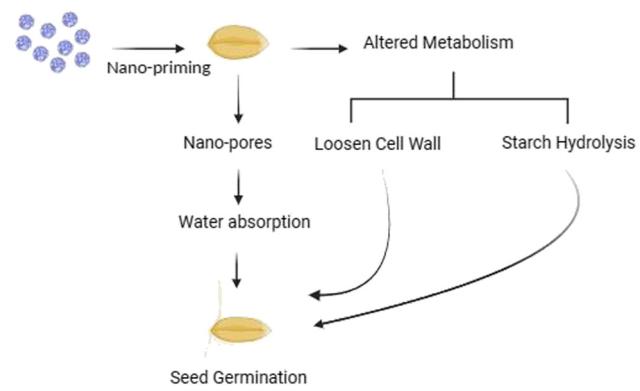


Figure 2: Effect of nanoparticles on seed germination.

are called PGPR, which are crucial in enhancing overall plant vitality [18]. PGPRs consist of a wide array of groups, including *Acinetobacter*, *Aeromonas*, *Agrobacterium*, *Allorhizobium*, *Arthrobacter*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Frankia*, *Gluconacetobacter*, *Klebsiella*, *Mesorhizobium*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, *Thiobacillus*, and others [19].

As the primary microbial community in the rhizosphere, PGPRs play a pivotal role in promoting plant growth. They function as biofertilizers, aiding in biotic and abiotic stress tolerance while enhancing plant nutrition, thereby facilitating the growth and development of host plants. These helpful bacterial groups protect plants and encourage their growth through various processes, including root colonization, favorable effects on plant physiology and growth, induced systemic resistance, and the suppression of phytopathogens. Traditional classifications of the methods by which PGPRs stimulate plant growth include direct activities occurring inside the plant and indirect actions occurring outside the plant [16].

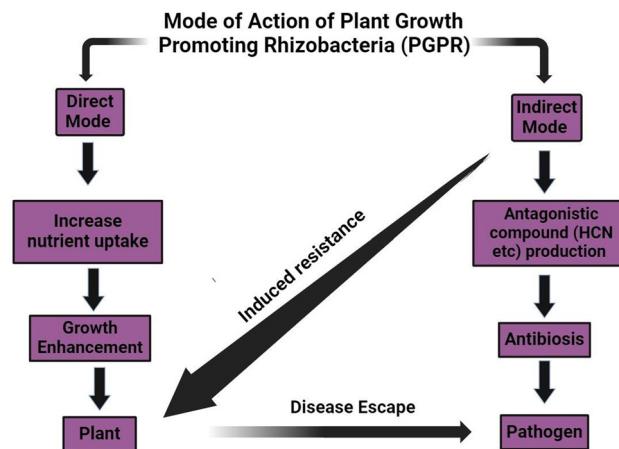
In direct mode of action PGPR fix nitrogen and solubilize minerals in roots vicinity (P, K, Zn, and Fe) that improves plant growth. They also regulate the amounts of phytohormones, including auxins, cytokinins, gibberellins, abscisic acid, and ethylene, which encourage plant growth and development [20]. PGPR indirectly improves plant abiotic stress resistance by suppressing phytopathogens and harmful microorganisms through parasitic actions, vying for resources and habitat in the rhizosphere, producing antagonistic compounds (such as HCN, siderophores, antibiotics, and antimicrobial metabolites), and producing lytic enzymes like chitinases, glucanases, and proteases. PGPRs protect plant health indirectly. Additionally, they cause plants to develop systemic resistance to various foliar and root diseases (Figure 3) [21].

## 2 Nanobiotechnology and PGPR mechanism in cold stress

The use of nanobiotechnology and bacterial strains modulate various mechanisms in plants. The details of these pathways are as follows.

## 2.1 NPs and cold adaptations

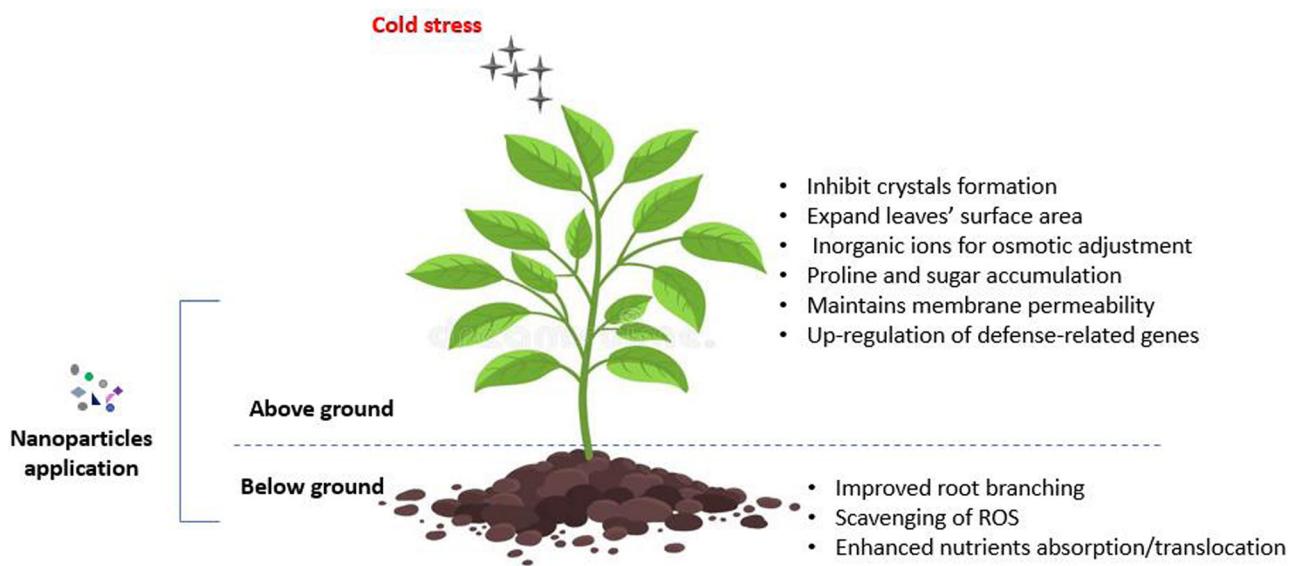
Chilling stress decreases plant productivity, and its detrimental consequences should be minimized. The unique



**Figure 3:** Role of plant growth promoting rhizobacteria on plants.

qualities of NPs (such as their tiny size, high mobility, biocompatibility, low cost, and increased reactivity) make them suitable for use in plants [22,23]. The use of NPs reduces the intracellular formation of ROS in a time-dependent manner, resulting in regulated electrolyte leakage and suppression of lipid peroxidation. There is a direct relationship between oxidative stress and the production of antioxidants. The activities of peroxidase, glutathione peroxidase, catalase, ascorbate peroxidase, lipoxygenase, and polyphenol oxidase are enhanced when plants face stress (Figure 4). Under cold stress, the use of NPs also maintains the level of photosynthetic pigments such as carotenoid, chlorophyll a, and chlorophyll b. They strengthen plant defense systems and aid in rapidly recovering plants exposed to cold stress [24]. NPs minimize the impacts of cold stress on plants by reducing the number of free radicals generated by plastids, increasing the rate of light absorption and electron transport in the chloroplast, and improving rubisco carboxylation. They increase the activity of enzymes in photosynthetic processes. Carbon nanotubes improve photosynthetic pigments in plants subjected to cold stress. They enhance the rate of electron transport in the chloroplast and reduce the production of free radicals. They upregulate the water channel genes that make the water available for photosynthesis even under a limited water supply [25,26] (Table 1).

Applying silicon NPs disrupts seed dormancy and increases germination characteristics in plants facing cold stress. They boost the seedling growth and biomass of *Agropyron elongatum*. Seed germination rates can be improved by 85–86%, which can be linked to changes in metabolic and physiological activities that result in earlier germination [34]. The complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) is an appropriate method for identifying stress-responsive genes. Amini *et al.* [35] investigated the effect of titanium dioxide NPs on *Cicer arietinum* facing



**Figure 4:** Role of nanoparticles on plant adaptations facing cold stress.

**Table 1:** Effect of cold tolerant bacterial strains on plants under low temperature

| Bacterial strain   | Plant                       | Mode of action   | Ref. |
|--|-----------------------------|--|------|
| <i>Lysinibacillus fusiformis</i> strain YJ4 and <i>Lysinibacillus sphaericus</i> strain YJ5                        | <i>Zea mays</i>             | ✓ Induced resistance against oxidative and osmotic stress<br>✓ Regulated phenolics, antioxidants, and phytohormones  | [27] |
| <i>Pseudomonas mosselli</i> , <i>Paenibacillus rigui</i> , <i>Paenibacillus graminis</i> and <i>Microvirga</i> sp. | <i>Oryza sativa</i>         | ✓ Improved growth in a genotype-dependent manner in cold stress<br>✓ Defense against phytopathogens<br>✓ Upregulated peroxidase genes that detoxify antioxidants | [28] |
| <i>Serratia nematodiphila</i>  | <i>Capsicum annuum</i>      | ✓ Enhanced abscisic acid and reduced salicylic acid and jasmonic acid  | [29] |
| <i>Burkholderia phytofirmans</i>   | <i>Vitis vinifera</i>       | ✓ Enhanced the osmolytes accumulation, <i>i.e.</i> , trehalose and trehalose-6-phosphate, that modulate cold stress.   | [30] |
| <i>Bacillus</i> strains RJGP41 and GBAC46  | <i>Solanum lycopersicum</i> | ✓ Volatile organic compounds enhanced plant growth   | [31] |
| <i>Pseudomonas</i> sp.   | <i>Triticum aestivum</i>    | ✓ Phosphate solubilization<br>✓ Improved NPK content in grains<br>✓ Enhanced grain yield and biological yield  | [32] |
| <i>Bacillus methylotrophic</i>   | <i>Solanum lycopersicum</i> | ✓ Improved the rate of photosynthesis<br>✓ Combat cold damage by enhancing antioxidant activities<br>✓ Alleviate cold shock reaction<br>✓ Improved fruit quality | [33] |

cold stress. They looked at 4,200 transcript-derived fragments and found that 100 were differentially expressed. After cloning 60 differentially expressed fragments, 11 of them produced readable sequences. These genes regulate various processes like cell connections and signaling, cellular defense, chromatin architecture, metabolic pathways, and transcriptional regulation. They have a gene–gene connection in response to cold stress. They play a role in cold tolerance and share a pattern with the results of cDNA-

AFLP. The production of these transcript-derived fragments due to the use of NPs at the outset of cold stress is critical to developing cold tolerance in plants. Elsheery *et al.* [36] found that foliar application of graphene nanoribbons, zinc oxide nanoparticles, selenium NPs, and silicon dioxide NPs on *Saccharum officinarum* reduced the effects of chilling stress and maintained a maximum level of photo-oxidizable PS I and photochemical efficiency of PS II. At the same time, carotenoid accumulation in the leaves increased non-

photochemical quenching of PS II. These findings indicated that silicon NPs have a strong ameliorative potential when compared to other treatments and can be utilized to improve plant productivity.

### 2.1.1 Chitosan NPs

Chitosan is a polymer made from chitin polysaccharides. Chitosan NPs are inexpensive, environmentally beneficial, biodegradable, non-hazardous, and have high solubility. They cause the buildup of osmoprotectants such as proline, carbohydrates, and amino acids. They decrease the number of ROS and the level of malondialdehyde in plants [37,38] – Chitosan NPs, combined with phenylalanine, aid in preserving fruit quality in *Diospyros virginiana* during cold stress. The treated plants have higher diphenyl-2-picrylhydrazyl radical scavenging activity than the control. In fruits, catalase, superoxide dismutase, ascorbate peroxidase, and ascorbic acid accumulation are elevated [39].

### 2.1.2 ZnO NPs

Chilling stress decreases chlorophyll production and chloroplast development, resulting in reduced photosynthesis. ZnO NPs are used to restore chlorophyll production and modulate the expression of cold-responsive genes in plants. They also reduce ROS generation and buildup. They are also helpful in preserving veggies at 4°C and ensuring their quality. They increase shelf life by maintaining product weight and membrane stability while lowering phenolic content. They have antimicrobial characteristics, which reduce the likelihood of infection [40].

### 2.1.3 TiO<sub>2</sub> NPs

TiO<sub>2</sub> NPs modulated gene expression in signal transduction, regulation, metabolism, and defense. They increase the expression of chilling-responsive genes while decreasing the plant damage index [19]. They mitigate the negative consequences of oxidative stress by inhibiting the formation of hydrogen peroxide and malondialdehyde. Increased levels of phenolic compounds also serve to neutralize ROS. They improve photosynthesis by increasing the capacity of plants to absorb sunlight and convert it into active electrons. Plants treated with TiO<sub>2</sub> NPs have higher carbohydrate, soluble protein, and soluble sugar content. Soluble proteins inhibit ice crystal formation by lowering free water availability in the intrinsic membrane [41]. According to Mohammadi *et al.* [42],

using TiO<sub>2</sub> NPs reduced electrolyte leakage, malondialdehyde concentration, and the membrane damage index in *Cicer arietinum*. Electrolyte leakage was reduced by 21–30%. TiO<sub>2</sub> NPs stimulate defense-related pathways in *Cicer arietinum*, resulting in cold stress tolerance. They activate antioxidants and osmolyte production and also upregulate defense-related genes. The accumulation of osmolytes decreases the freezing point and reduces the damaging effects of stress. They influence metabolic processes, which in turn influence physiological and biochemical processes. Phosphoenolpyruvate carboxylase is a carbon fixation enzyme that also plays a role in metabolic adaptations. Its high activity is involved in creating malate and other cold-tolerant metabolites. The application of TiO<sub>2</sub> NPs improves the activity of phosphoenolpyruvate carboxylase that generates malate as an osmolyte, which causes cold tolerance in plants [43].

## 2.2 PGPR and cold adaptations

Worldwide crop production is negatively impacted by abiotic stressors. Abiotic stresses include cold stress, a significant plant stress that can reduce crop production by causing physiological and metabolic imbalances, resulting in the accumulation of ROS, nutritional problems, membrane dysfunction, lowered photosynthetic capacity, and hormonal imbalance [44]. It has been extensively observed that PGPR treatment can reduce abiotic stress in plants. PGPR improves stress tolerance by stimulating antioxidant, hormonal, photosynthetic, and other stress-related pathways in plants [45].

### 2.2.1 *Bacillus* sp.

*Bacillus* strains are spore-forming, gram-positive, plant-colonizing, and growth-promoting bacteria that belong to the PGPR group. Due to their inherent rigidity and durability, *Bacillus* is a functionally varied group of microorganisms that can create a wide range of metabolic products and endure extreme environmental conditions [46]. The existence and expression of specific genetic characteristics in the *Bacillus* genome that allow them to exhibit such physiological responses are responsible for their capacity to withstand stress. The ability of *Bacillus* to tolerate cold stress is commonly linked to the gene families associated with cold shock proteins, signal transduction pathways, oxidative stress and antioxidant enzymes, osmotic control, and membrane transportation. By regulating crucial signaling pathways, the expression of these gene families

causes specific transcriptional modifications that improves water transport across their membrane, maintain water balance inside the cell, and improve sensitivity to cold stress. In addition to all of these crucial genetic traits and their byproducts, biofilm formation is an essential factor for the *Bacillus* to colonize and create a variety of vital secondary metabolites in the face of challenging environmental circumstances [47]. Wang *et al.* [38] demonstrated that application of consortium of *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21 on tomato seedlings enhanced its ability to tolerate chilling stress at 4°C. The *Bacillus amyloliquefaciens* strain NBRI-SN13, also known as SN13, characterized for a variety of plant growth-promoting characteristics and stress tolerance, including auxin and ACC deaminase production, solubilization of tricalcium phosphate, and proline accumulation under salt stress [48].

### 2.2.2 *Pseudomonas* sp.

*Pseudomonas* sp. improves cell elongation and proliferation by IAA production and decrease the synthesis of ethylene by ACC deaminase production in plants facing unfavorable conditions. They also solubilize phosphorus and sequester iron the rhizosphere. improves plant growth under various environmental stresses. By reducing the expression of ETF-13 and ACC oxidase in plants, *Pseudomonas* spp. protects plants against cold stress. Negi *et al.* [49] demonstrated the effect of *P. fluorescens* 173, *P. fluorescens* 193, *P. fluorescens* 547, *P. fluorescens* 551, and *P. fluorescens* 572 on *Phaseolus vulgaris* to alleviate cold stress. Applying PGPR resulted in HCN and siderophore production and phosphate solubilization and promoted plant growth. *Pseudomonas vancouverensis*, when used on *Triticum aestivum* for alleviation of chilling stress, helped in tolerate a wide range of pH, solubilize tricalcium phosphate, indole acetic acid, HCN, and siderophore production [50]. In *Vigna radiata* under cold stress, application of CRPF2 (*P. fluorescens* strains GRS1 mutant) improves shoot and root length and formation of nodules because *P. fluorescens* have great ability to colonize the roots and high solubilization of phosphorus [51].

## 3 Screening of anti-freezing proteins

Anti-freezing proteins are a physically diverse class of proteins that inhibit the production of ice crystals inside and

outside the cells, as well as inhibit structural damage. They lower the freezing point of liquids and change the shape of ice crystals. Ice recrystallization inhibition and thermal hysteresis are features of anti-freezing proteins. These anti-freezing proteins have been successfully introduced in various plants, including *Solanum tuberosum*, *Solanum lycopersicum*, and *Nicotiana tabacum*. Colorimetric detection of anti-freezing protein activity has recently been introduced using nanobiotechnology, which offers an advantage over older methods. Antifreeze proteins' stability and concentration-dependent activity were detected using freeze-labile gold NPs. It is relatively easy, requires less labor, is less expensive, and has a four-fold faster screening time, making it more viable than traditional approaches [52].

When soil temperatures are low, fungus pathogens are nastier. Low-temperature tolerant (psychrotrophic) bio-control PGPRs have a greater chance of becoming more practical in cold environments [45]. Additionally, because spring is generally colder (*i.e.*, 5–10°C), PGPR should exhibit vigorous activity in places with very cold soil temperatures and be able to withstand cool-warm cycles, which are frequently observed. It has become more significant ever since it was discovered for the first time that several psychrophilic and psychrotrophic bacteria, notably PGPR, leak antifreeze proteins into the surrounding medium that protect the bacterial cell wall and membrane from the damaging effects of ice crystals. Bacterial antifreeze proteins also exhibit ice-nucleation activity, which appears to govern the generation of ice crystals outside of the bacterium. *Pseudomonas putida* GR12-2 at 5°C, improves root extension in both spring and winter canola. Additionally, the bacteria survived exposure to 20–50°C, both below freezing. *P. putida* GR12-2 produced and released a protein with antifreeze activity into the growth medium after growing at 5°C, which was shown to provide the molecular foundation for this behavior. The medium was run through SDS-polyacrylamide gel and it was found that the bacteria release one main protein having the weight of 32–34 kDa and several smaller proteins at low temperature [53]. Raymond *et al.* [54] research on the isolation and characterization of antifreeze proteins from cold-tolerant bacteria. They isolated a *Colwellia* strain SLW05, a gram-negative bacteria found in Antarctic Sea ice, creating an extracellular material that modifies the shape of developing ice. A 25-kDa protein that functions as an inhibitor of ice recrystallization to safeguard membranes in the frozen state was shown to be the active ingredient.

## 4 Fruits refrigeration/preservation

Fruits are high in antioxidants, phenols, and vitamins, which help prevent cancer and degenerative disorders. The fruit sector is dealing with the issue of storing fresh-cut fruit at low temperatures – browning effects after cutting and peeling impact fruits' texture and shelf life. Using  $\alpha$ -tocopherol nano-capsules maintains firmness, decreases browning effects, and extends the shelf life of fruits in cold storage. Nano-capsules are safe and uniformly cover the surface area, preventing oxygen interaction due to polyphenol oxidase activity. In contrast,  $\alpha$ -tocopherol has antioxidant potential that reduces the action of pectin of methyl esterase, resulting in fruit firmness [55,56]. Li *et al.* [57] investigated the effect of a nano-packing film incorporating a polylactic acid matrix with zinc oxide NPs on apple storage at  $4^{\circ}\text{C} \pm 1$  for 2 weeks. The findings suggest that nano-packing preserves sliced fruits' color, phenolic content, and hardness. They also limit microbial development and lower the browning index in storage. As a result, they can be utilized to improve the quality of preserved cut fruits. Pilon *et al.* [58] showed that chitosan NPs had excellent antibacterial action against psychrotrophic and mesophilic bacteria and inhibited yeast and mold formation on fruits under refrigeration. They extend the shelf life of cut fruits and can be used as an edible coating to limit microbial growth.

The adverse effects of chemical preservatives on human health create a space for the search for potential bio-preservatives for plant products. Some bacterial strains work efficiently against food-borne pathogens, including fungi, yeast, and bacteria. Lactic acid-producing bacteria release metabolites that neutralize mycotoxins. The lactic acid-producing bacteria show antibacterial properties due to the synthesis of bacteriocins, hydroperoxide, organic acids, and lactic acids. It is an alternative strategy for using chemical preservatives. The criteria for an excellent bio-preserved include high activity, the release of non-toxic metabolites, safety in use, and lack of any negative impact on food. Lactic acid bacteria are broad-spectrum against unfavorable microflora. Food products fermented with lactic acid bacteria also have health benefits [59].

linked to these alterations. Plants can be significantly helped by nanobiotechnology and bacterial strains in cases of cold stress. They control osmotic balance, gene expression, and other metabolic functions. NP and plant growth-promoting rhizobacteria application upregulates cold-responsive genes, which can be identified and exploited to create transgenic plants with enhanced cold tolerance. They also activate anti-freezing proteins and help to maintain fruit quality and shelf life in storage.

The recommendations for future perspective are as follows;

- 1) Cold-resistant plant growth-promoting bacterial strains should be used to develop efficient biofertilizers.
- 2) Studies should be carried out at the molecular level to identify possible mechanical changes in plants that could be used to develop cold-tolerant transgenic plants.
- 3) Nanobio-formulations can be synthesized for sustainable growth of crops under chilling stress, and their safety limit should be assessed.
- 4) Proper bio/organic methods should be designed to preserve the fruits and extend their shelf life.

**Funding information:** The authors of this review article are truly thankful for research support funds for two projects: 1) Study on information of ecological protection in world's great river basins (Grant # K23036Y), and 2) Henan Provincial Funding for the Construction of Key Laboratory of Soil Ecology and Environment (Grant # J23068Y). The authors are also grateful for the financial and moral support of College of Geography and Environmental Sciences, Henan University Kaifeng, and Department of Botany, PMAS Arid Agriculture University Rawalpindi, Pakistan for the collaborative research work.

**Author contributions:** Authors Nosheen Akhtar and Asim Shahzad contributed equally to this work and should be considered first co-authors. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Conflict of interest:** The authors state no conflict of interest.

## 5 Conclusion and future perspective

Cold stress disrupts cellular metabolism and alters photosynthesis, respiration, mineral nutrition availability, and water regime. The expression of transcription factors is

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