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Recent advances in PGPR and molecular mechanisms involved in drought stress tolerance

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Abstract

Increased severity of droughts, due to anthropogenic activities and global warming has imposed a severe threat on agricultural productivity ever before. This has further advanced the need for some eco-friendly approaches to ensure global food security. In this regard, application of plant growth-promoting rhizobacteria (PGPR) can be beneficial. PGPR through various mechanisms viz. osmotic adjustments, increased antioxidant, phytohormone production, regulating stomatal conductivity, increased nutrient uptake, releasing Volatile organic compounds (VOCs), and Exo-polysaccharide (EPS) production, etc not only ensures the plant's survival during drought but also augment its growth. This review, extensively discusses the various mechanisms of PGPR in drought stress tolerance. We have also summarized the recent molecular and omics-based approaches for elucidating the role of drought responsive genes. The manuscript presents an in-depth mechanistic approach to combat the drought stress and also deals with designing PGPR based bioinoculants. Lastly, we present a possible sequence of steps for increasing the success rate of bioinoculants.

Keywords:

PGPR, Global food security, Sustainable agriculture, Omics techniques, Bioinoculants

1. Introduction

Various abiotic stresses are increasing in magnitude due to frequent climate changes and anthropogenic activities, among which drought stress is particularly challenging. Drought is a climate-specific term characterized by less than normal or no precipitation within a given time and is broadly categorized into 4 types viz. meteorological, agricultural, hydrological, and socio-economic drought (1). About one-third proportion of the net land area falls into arid and semi-arid regions, though sudden meteorological drought is prevalent in other normal regions. Lack of adequate water supply not only proves fatal to plants but also causes massive societal problems and yield losses (Table 1). Drought is likely to put severe plant growth constraint for about one half of the cultivable lands by 2052 (2). Majorly our food relies on cereals and pulses which need excess water during their cultivation. Drought incidence in such crops can bring serious agricultural damage and can negatively affect global food security. For that reason, the scarceness of water resources has been reflected as a critical worldwide environmental problem. Droughts have begun to intensify on a global scale, disrupting both ecosystem framework and functioning. Especially, in arid and semi-arid areas, adverse climatic incidents such as droughts are expected to become more severe, more rapid, and of longer duration (3). As per the report of the Intergovernmental Panel on Climate Change (IPCC) 2018, industrialization has raised the global mean temperature by 1.0 °C. It was also indicated that, by 2052, maintaining anthropogenic activity at the present level could raise the global temperature by 1.5 ° C (4).

Consequently, new strategies are needed more than ever, to uplift production so as to fulfill global food requirements. Extensive studies and policy build-up are being carried out globally to muddle through drought stress. Development of drought-tolerant varieties, practicing better resource organization, changing the crop calendars, etc are some of the significant strategies used in combating drought. The integration of microbes as a part of cultivation system can be yet another significant as well as eco-friendly strategy against drought (5, 6) economic and sustainable way to uplift production during extreme water deficit. Inside the soil environment, plant-microbe relations are decisive in maintaining primary productivity. Integrating microbes is an effective approach in drought stress mitigation. Up to 41% yield increase was shown in wheat plants after applying a consortium of PGPR and arbuscular mycorrhizal fungi (AMF) (7). Similarly, 40% root enlargement and increased tolerance to water deficit, was observed in pepper plants primed with PGPR (8). Likewise, many other studies highlighting rhizobacterial role in drought stress amelioration were done in peppermint (9), maize (10), foxtail millet (11), guinea grass (12), and pulses (13). However, under severe drought, which is often accompanied by critically low soil water content, PGPRs help plants to survive but fails to maintain crop productivity.

In this review, we are therefore seeking to shed more light on the various mechanisms employed by bacteria to ameliorate the harmful impacts of drought stress on plants and further using them for agricultural sustainability (14).

Table 1- Economic yield reduction by drought stress in some representative field crops

Crop	**Yield reduction (%)**	**Reference**
Barley	49–57	(15)
Maize	79–81	(16)
Wheat	57	(17)
Rice	53–92	(18)
Chickpea	45–69	(19)
Pigeonpea	40–55	(20)
Soybean	46–71	(21)
Sunflower	60	(22)
Potato	13	(23)
Common beans	58–87	(24)

2. Drought adversities in plants

Since it is concerned with tissue metabolism, the relative water content (RWC) of leaves is thought to be a good parameter for calculating plant water status. The actual amount of soil water (which remains available for absorption by plants) is known as relative water content, which lies somewhere between field capacity (FC) and permanent wilting potential (PWP). Field capacity is represented by the moisture content left in the soil following gravitational pull, which is stored in the macropores, after being completely saturated with water and reflects the upper limit of available water. The permanent wilting point (PWP) on the other hand, is the matric potential at which plants are unable to pull out water content from the soil. Apart from RWC, drought effects can be studied via various plant parameters like the total anhydrous leaf weight, total anhydrous stem weight, leaf area index, the total number of nodes present, quality of plant fiber, the canopy of plant, and its root development (25, 26). However, its generalized effects rely upon various factors like plant species and their genetic constitution, the size, and age of the plant along with the period and stringency of stress (27). Dought can affect various sub-cellular compartments, cell organs, and whole plant levels (28). Water deficit can interfere with seed germination in the early stages of plant development and slows down the plant growth in later stages, by hampering cell elongation (29). Hence, plants are shorter, with lesser leaf area, absorbing lesser photosynthetically active radiations (PAR), thereby, limiting photosynthesis and yield. Nutrients are vital for the normal plant growth and nutrient transport is severely affected under drought as it disrupts the mass flow of water-soluble nutrients e.g. nitrate, sulfate, Ca, Mg, and Si. Drought can affect some crucial enzymes e.g. nitrate reductase (NR), caused by lesser nitrate absorbtion from soil (30). The first direct sign of drought in plants become evident from the photosynthesis rate, caused by decreased conductivity of stomata and a concomitant rise in photorespiration (31) . Moreover, many non-stomatal factors also affect the net photosynthesis such as a drop in catalytic activity of RuBisco, amount of CO_2 present in the chloroplast, and effectiveness of PSII photosystem (32). Closure of stomata can also trigger reactive oxygen species (ROS) based damage like lipid peroxidation, elevated malondialdehyde (MDA) level, loss of membrane integrity, and degrade protein, lipids, and nucleic acids (33) . Oxidation of photosynthetic machinery frequently causes a decline in chlorophyll level under drought. It also heightens the stress ethylene stature in plants, restraining growth of the plant by different means (34). Drought stress also influences carbohydrates, energy level, respiration, and abscisic acid (ABA) level in the cell (35). Drought can become more detrimental during certain stages of plant growth e.g. if drought abrogates photosynthesis during anther development, it can cause male sterility as pollen maturation ceases due to an insufficient level of carbohydrates inside the cell (36). Thus, drought negatively affects both quantity as well as the quality of growth in plants. Therefore, to ensure global food security, drought stress mitigation is highly desirable.

3. Mechanistic outlook of PGPR in drought stressed plants

Several mechanisms are known so far, which enable microbes to exert tolerance towards drought in plants, including alteration in osmotic tuning, antioxidant machinery, plant hormonal level, stomatal conductivity, nutrient uptake, release of volatile organic compound (VOC), and exo-polysaccharide production (EPS) (Figure 1). Besides these, the synergic application of plant growth promoting rhizobacteria (PGPRs) and silicon (Si) also helps in sustaining the physiological fitness of plants for improved carboxylation linked with the increased growth rate in various abiotic stresses like salinity and drought (37).

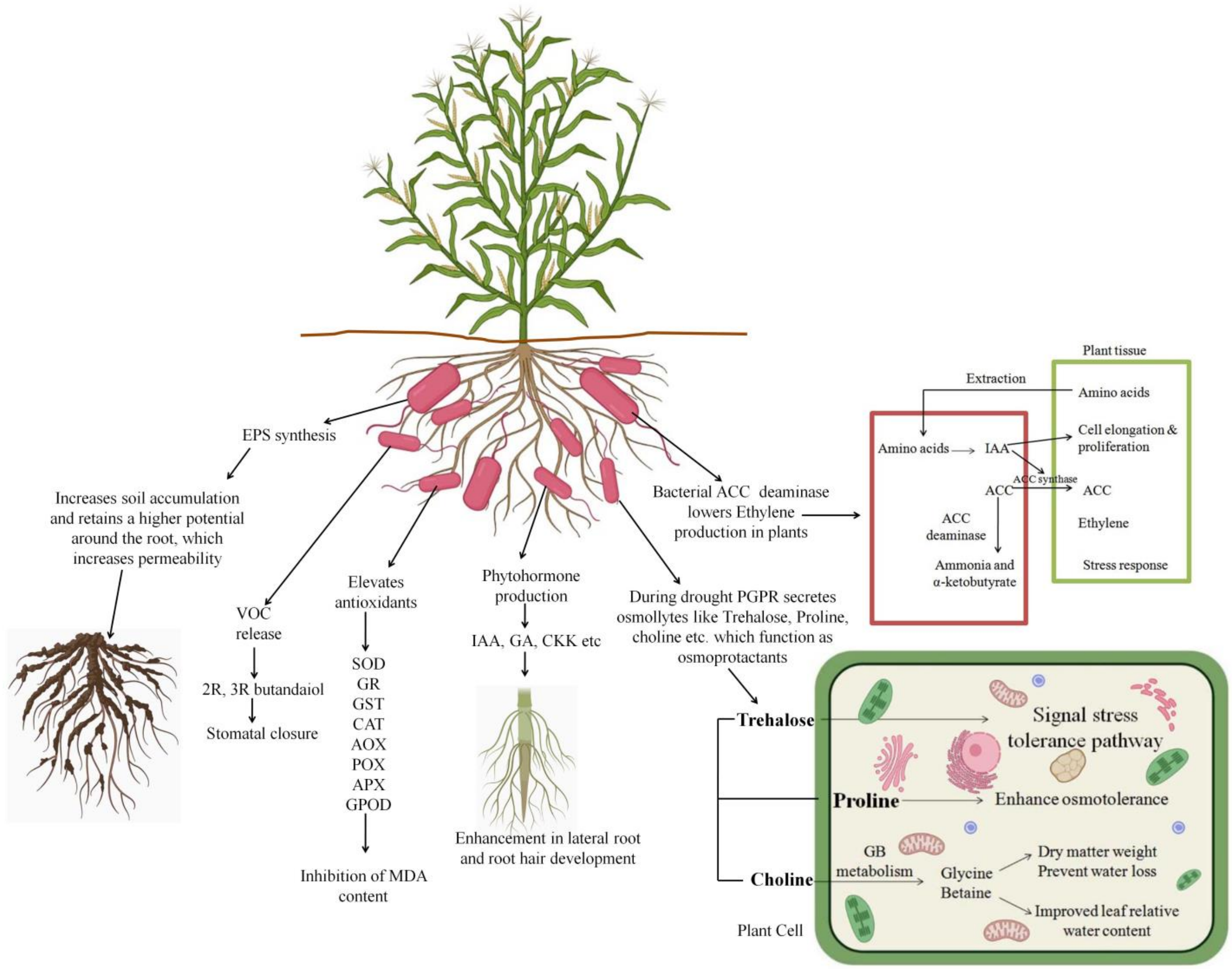

Fig 1- Modulation in EPS, VOCs, Antioxidants, Phytohormones, Osmolytes, ACC deaminase are some of the mechanisms of PGPR used in the mitigation of drought stress in plants. (**Abbreviations**: **EPS**- Exo-polysaccharide, **VOC**- Volatile organic compounds, **SOD**- Superoxide dismutase, **GR**- Glutathione reductase, **GST**-Glutathione S-transferase , **CAT**- catalase, **AOX**- Alternate oxidase, **POX**- Peroxidase, **APX**- Ascorbate peroxidase, **GPOD** – Guaiacol peroxidase , **MDA**- Malondialdehyde, **IAA**- Indole acetic acid, **GA**- Gibberlin, **CKK**- Cytokinin, **ABA**- Abscisic Acid, **ACC**- 1-aminocyclopropane-1-carboxylate **)**

3.1 Osmotic tuning & turgor pressure re-establishment

Osmolytes synthesized by rhizobacteria under drought conditions can enhance the growth of the plant (38). Proline , a crucial osmolyte and can be accumulated during drought condition in plants and owing to its hydration potential, it can easily bind to proteins, making them soluble and thus averting their chances of denaturation. Proline also regulates the redox potential of cells and curtails cell acidity by neutralizing free radicals. When a dehydrated cucumber plant was inoculated with a consortium of *Bacillus subtilis*, *B. cereus*, and *Serratia* sp., a 4-fold increase in proline level was observed (39). Various recent studies suggest a positive correlation between the drought stress tolerance of the plants and their proline content e.g. proline level improved under drought in garden pea (40, 41), chickpea (42), rice (43), and soybean (44). On the molecular level , Δ1-pyrroline-5-carboxylate synthetase (P5CS) and PROLIN DEHYDROGENASE (ProDH) are 2 major genes that adjust proline formation (45). Trehalose is a reducing disaccharide that prevents solidification of the lipid bilayer and also secures other cellular machinery and proteins during drought. It is synthesized by the trehalose-6-phosphate synthase gene (46). Also, betaine a water-soluble alkaloid quaternary ammonium compound (47) aids in strengthening the photosynthetic apparatus, by elevating the synthesis of protective enzymes, and stabilizing membrane integrity under drought. Polyamines like cadaverine, spermine (Spm), spermidine (Spd), and putrescine (Put) are additional osmolytes improving osmotic tolerance during drought stress. Thus, advanced portrayal of crucial genes regulating the synthetic and metabolic pathways of these compatible osmoprotectants are to be characterized on the genetic level, for further improving drought stress endurance in plants.

3.2 Protection by improved antioxidants stature

Drought is often followed by ROS overproduction due to elevated photorespiration rate and damaged photosynthetic components. ROS like superoxide ions, hydrogen peroxide, hydroxyl ions, and singlet oxygen also interfere with the 3-D arrangement of membrane proteins, making them more permeable and causing ion escape, chlorophyll impairment, metabolic distress, and severe damage and eventually plant's death. To quench excess ROS, plants have evolved an antioxidant system, having both non-enzymatic and enzymatic components. Its main enzymatic components include glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), , alternative oxidase (AOX), peroxidase (POX), , guaiacol peroxidase (GPOD), ascorbate peroxidase (APX) etc and non-enzymatic groups include various reductants like glutathione (GSH), cytochrome f (Cytf), ascorbate (AsA), flavonoids, carotenoids (CAR), anthocyanins, tocopherol, (Vit E), etc. Even though plants are well equipped with antioxidant defense response, plant productivity sternly declines during water deficit. Many studies suggested a strong link between stimulation of antioxidant response and level of drought endurance. Potato plants primed with two PGPR strains, *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40, triggered an upsurge in ROS-quenching enzymatic components involving ascorbate peroxidase and catalase. Similarly, the specific enzyme activity of numerous ROS-quenching enzymes comprising APX, CAT, and SOD was found escalated in PGPR primed plants (48). A similar correlation of drought endurance and CAT profile was shown in cucumber (49) , maize (50), and wheat (51). However, some other studies focused on the assessment of enzymatic antioxidants to investigate the ROS damage that occurred during drought conditions. Lipid peroxidation caused by ROS damage can be evaluated by MDA content. PGPR-treated cucumber exhibited declined MDA level and reduced electrical conductance in leaves for uninoculated plants. These studies clearly illustrate the function of enzymes as ROS quenchers and how PGPR stimulate the oversupply of enzymatic antioxidant gadgetry in plants, to exert drought endurance.

3.3 Modulating phytohormonal level

Various chemical regulators are capable of modulating the plant's development and growth. These are better known as phytohormones and comprise a diverse range of chemicals including auxins, cytokinins, abscisic acid, gibberellins, and ethylene, etc. By changing the plant hormone level, PGPR imparts drought resistance in plants. Water deficit triggered by drought causes photosynthetic drop and badly affects the root metabolism. PGPR inoculated plants initiate an extensive reprogramming in root system architecture (RSA) involving increase in the root tips surface area (52) therefore enhancing nutrient and water transport under stress.

Auxins, also known as indole-3-acetic acid (IAA), has a central role in monitoring the development of plant by controlling a broad range of metabolic functions e.g. differentiation of xylem and phloem, primary and secondary root development, promoting division in cells, elongation of root and shoot and their tropic movements. There are various studies reporting auxin production by rhizobacteria primed crop plants (53) e.g. wheat plants inoculated with *Azospirillium* by endogenously modulating IAA, improved the water stature in leaves (54). Another study in *Bacillus thuringiensis* briefed wheat plants confirmed elongation of root hairs and showed remarkable improvement in root hair density by auxin and ACC deaminase (ACCd) synthesis by rhizobacteria. These results are in synchronization with previously reported work, suggesting about the improved IAA level by a consortium of *Bacillus megatherium* and *Pseudomonas putida* in clover plants (55), *Phyllobacterium brassicacearum* in *Arabidopsis* plants (56) and *B. thuringiensis* in *Lavandula* plants (57). The common IAA-producing bacteria *Azospirillum*, *Bradyrhizobium*, and *Pseudomonas* can encourage root development (58).

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Similarly, Gibberellins (GA) are another class of plant hormone having key function in seed germination, elongation of the stem, flower onset, fruit ripening, and senescence. GA synthesizing bacteria help in water stress tolerance when briefed to the host (59). In a study, when bacterium *Azospirillum lipoferum* was primed onto maize plants, a considerable augmentation in GAs level was observed (60). There are many earlier reported studies suggesting about the increased GA level after inoculating with rhizobacteria e.g. using consortium of *Acinetobacter calcoaceticus* SE370 , *Burkholderia cepacia* SE4, and *Promicromonospora* spp. SE188 on cucumber plants and *Pseudomonas putida* on soybean plants (61). Common GA-producing rhizobacteria include *Acetobacter diazotrophicus*, *Azospirillum lipoferum*, *A. brasilense*, *Bacillus subtilis*, *B. cereus*, *B. licheniformis*, *Burkholderia* sp., *Herbaspirillum seropedicae*, and *P. fluorescens* (62). Cytokinins are yet another type of plant hormone responsible for stem growth, plant cell division, photosynthesis, and regulating stomatal opening under drought. The influence of cytokinins on plant development are well documented in PGPR treated host plants during drought conditions (63) . Besides many cellular roles, Abscisic acid (ABA) has a prominent function against many ecological stresses like drought (64). ABA is quickly synthesized within plants as a key anatomical reaction to drought. Its rapid synthesis begins inside roots of plants, and further gets transported to leaves; whereby regulating the opening and closure of stomata, improving plants drought endurance. *Azospirillium lipoferum* inoculated maize plants augment relative water content and turgidity by ABA production. Up to 4% and 10 % decrease in water loss have been reported from grape leaves when treated with *B. licheniformes* and *P. fluorescens*, respectively (65).

Throughout drought stress, root maturation and development of plants are badly influenced due to up-regulated Ethylene (ET) synthesis in plants. The improved amount of ET generated as a reply to different abiotic and biotic stresses is termed stress ethylene (66) . This sudden upsurge of ET above a critical level can cause several inhibitory consequences on plants, like prohibiting seed germination, disrupting root development, leading to senescence. Some studies documented that IAA has a role in reducing ET level and hence promoting root development in PGPR primed plants (67). PGPR inhabit the seed or root region of developing plants, from where they gain Tryptophan and similar molecules in seed or root exudates and further metabolize them into IAA and release into the rhizoplane. IAA derived from bacteria, along with the plant generated IAA can either promote plant growth or up-regulate the synthesis of ACC synthase, a plant enzyme that catalyzes the conversion of S-adenosyl methionine (SAM) to 1-Aminocyclopropane-1- carboxylate (ACC). ACC acts as the predecessor of ET in higher plants. Some fraction of this freshly formed ACC is released from seeds and roots into the rhizosphere and is transformed by PGPR into αketobutyrate and ammonia, by enzyme ACC deaminase (ACCd) (68). This bacterial enzyme is important in preventing plants from the adversities of increased ethylene levels. Hence, by modulating the ACC level, PGPR implicates their benefits under several abiotic stress (69).

Under drought conditions, the growth enhancement in plants by several ACC deaminase positive rhizobacteria has been studied (70). For instance many earlier reports of priming plants with ACCd producing bacteria have been well documented in pea (71), pepper and tomato (72). Likewise, the briefing of *Bacillus licheniformis* K11in pepper plants resulted in improved drought endurance (73). Plant biomass enhancement and better root and shoot morphology during drought was observed in velvet beans. Reduced discharge of ethylene in roots and leaves of velvet beans was accountable for the observed effects (74). Treatment of ACCd positive and EPS producing, *Pseudomonas fluorescens* DR7 in foxtail millet showed better adhereance of soil aggregates to root tissue and also increased soil dampness (11).

Salicylic acid (SA) has also been important in plant defense and hence plays a significant role in drought endurance (75). The doubled SA level in barley roots was accountable for improved tolerance under drought (76). By triggering the activation of various genes like PR ,WRKY and TGA, , SA helps the plant to better cope up with various stressors (77). Similarly, jasmonic acid (JA) successfully advances the plant drought endurance by reinforcing enzymatic antioxidants and increasing the activity of organic osmoprotectants (78).

To conclude, phytohormones have immense potential and are crucial for achieving the target of drought-surviving crops. Further plant's reaction towards drought can be altered by decoding their complex interplay and by increasing or decreasing the regulation of phytohormone-related genes.

3.4 Attuned Stomatal conductivity

Drought onset reduces soil water content, leading to the closing of stomata thereby affecting the photosynthesis rate; conversely priming of plants with PGPR augments the photosynthesis rate by enhancing water level, greater CO_2 now disperses in the mesophyll area due to extended stomatal conductivity. The stomatal retort to drought occurs by two means: (1) by sensing the air moisture level where guard cells & nearby epidermal cells bring on the closure of stomata by directly dissipating humidity, (2) by replying to changes in the water potential of leaf, where stomatal closure occurs after water potential value drops below a certain critical level. K^+ is a major regulatory factor governing the opening and closure of stomata and its directionality inside guard cells controls the water potential and turgor pressure. The cell membrane of guard cells is placed with numerous protein complexes (e.g. acetylcholine receptors, ABA-binding proteins, light receptors, and GTP-binding proteins), channels, and pumps for smooth trans-membranal K^+ transport. During drought, stomatal closure helps in water preservation in plants. Bacterial mediated stomatal movements helped in improved stomatal regulation e.g. the closing of stomata was also observed in *Pseudomonas chlororaphis* O6 primed plants regardless of the plants ABA stature (79). Similarly, when both normal and ABA- deficient *Arabidopsis* were inoculated, reduced stomatal opening was observed leading to, reducing transpirational loss and thus delivering drought endurance. Prolonged water stress conditions often generate free radicals and ROS, which irreversibly harm the photosynthetic apparatus after reacting to them. Rhizobacteria priming can be useful under such conditions since it elevates plant water level and enhances photosynthesis by escalating stomatal conductivity. However, further investigation is needed for a clear picture of the exact pathways which trigger the microbial regulation of the stomata movement in plants under water deficit.

3.5 Enhanced nutrient uptake

Water deficit during drought impedes the movement and nutrients diffusion inside the soil due to compromised soil structure. Several reports are indicating enhanced nutrient acquisition in plants when inoculated with beneficial rhizobacteria. For instance, *Bacillus thuringiensis* when inoculated with *Lavandula* plants under drought led to a sharp rise in both macro-(Ca^{2+}, Mg^{2+}, and K^+) and micronutrients (Cu^{2+}, Mn^{2+}, and Zn^{2+}) in the stem region of the plant. Rhizobacteria-treated *Cucumis* plants when exposed under drought stress demonstrated improved K and P ion content. Likewise, inoculation of *Variovorax paradoxus* 5C-2 promoted nodule formation in roots of pea and the effect was reflected as higher Nitrogen level in leaves (80). Similarly, augmented K^+ levels caused by an autochthonous bacterium in indigenous *Lavandula* and *Salvia* plants species, eventually caused reduction in stomatal conductance essential for maintaining turgor pull under severe drought conditions. In this perspective, concomitant inoculation of PGPR and Arbuscular Mycorhizza (AM) in plants is also an effective approach in coping severe drought, e.g. K^+ content in *Trifolium* plants escalated up to 2.17 times on treating with a blend of AM and *Bacillus thuringiensis* and goes further up to 3.48 times in *Pseudomonas putida* and AM treated plants. Apart from this, a sharp elevation in the micronutrient level was also recorded (81). A similar report on the comparative efficacy of AM and PGPR reflected that *Klebsiella* and *Bacillus* treated Lettuce plants showed more P, K content and greater biomass than AM treated ones (82). Additional research on rhizobacterial-driven nutrient enhancement of plants may pave the way to bio-fortification in an environment-friendly manner, along with increasing the green cover and reducing the global carbon footprint.

3.6 Release of Volatile Organic Compounds (VOCs)

Bacteria derived volatile organic compounds are generally comprised of lipid-soluble liquids with high vapor pressure. Their synthesis is species-specific and takes part in inter-specific communication, cell signaling, and growth enhancement. Various reports have drawn attention about the growth promotion by rhizobacteria based VOCs. 2R, 3R-butanediol formed by *Bacillus amyloliquefaciens* IN937a and acetoin by *Bacillus subtilis* GB03 increased growth in *Arabidopsis* by altering the expression of cell wall structure governing genes (83). It was further accounted that acetoin by *Bacillus subtilis* GB03 increased the transcription rate of auxin and also triggered production of osmolyte glycine betaine and choline exerting drought resistance. A similar study involving VOC compound, 2R-3R-butanediol, synthesized by *Pseudomonas chlororaphis* O6 suggested that by closing stomata in Arabidopsis, it imparts drought tolerance to host plant (84). Plants also produce VOCs in very limited amounts and continually release them from leaves. However, their synthesis is up-regulated during stress which leads to a sharp fall in the photosynthetically fixed carbon. These VOCs work as messengers to elicit stress-related defense responses.

3.7 Exopolysaccharide (EPS) fabrication

EPS fabrication by rhizobacteria is a crucial characteristic for growth enhancement during drought as it helps in forming hydrophilic biofilms while inhabiting the roots of the plant, thus shielding them from soil dehydrating conditions (85). Bacterial EPS is generally composed of homo- or hetero- polysaccharides which are held to cell surface as a slime layer or capsule and constitutes biofilms. The constituents of polysaccharide differ among distinct PGPR however general monomers contain glucose, galactose and mannose. These variable polysaccharide components are responsible for difference in water retention capacity of various PGPR and it may reach up to 70 gm water per gram of polysaccharide (86). The constituents and synthesis of EPS depends upon growth phase of bacteria, composition of medium in which bacteria is grown (carbon/nitrogen ratio) and ecological conditions of surroundings. Several reports have well documented the effects of EPS triggered drought endurance in PGPR primed plants like wheat (87), maize, and sunflower. *Azospirillum*, an EPS forming bacteria augmented drought tolerance via adjusting soil binding and structural parameters (88). Rhizobacteria generate EPS under drought conditions, which lead to proper root development, better shoot development and increase in net dry mass of plants. Thus, EPS works by binding the soil particles together and maintaining water level in rhizosphere. In *Psuedomonas aeruginosa*, water stress triggers transcription of *Alg* genes in the alginate biosynthesis gene cluster which are prominent in eliminating drought stress (89). Similar alginate-triggered drought stress endurance was seen in wheat primed with *B. thuringiensiss*. Similarly, stress environment induces guanine cyclase synthesis in cells which in turn causes EPS production (90) (Table 2).

Table 2- A compilation of rhizobacterial effects on drought-stressed plants.

Bacterial Strain or Consortium	**Inoculated Plants**	**Effects by PGPR**	**References**
Penibacillus polymyxa	*Arabidopsis thaliana*	Increase gene expressions related to abiotic stress (ERD15, RAB18)	(91)
Pantoea agglomerans	*Triticum aestivum*	EPS production	(92)
Rhizobium spp.	*Helianthus annus*	EPS production	(93)
Bacillus	*Lactuca sativa*	CKK production	(94)
Pseudomonas spp.	*Pisum sativum*	ACCd production, increase root weight & no. of pods	(71)
Rhizobium tropica *Paenibacillus polymyxa*	*Phaseolus vulgaris*	Increase nodulation & N content	(95)
Variovorax paradoxus	*Pisum sativum*	Decrease ethylene due to ACC deaminase production	(80)
Pseudomonas putida	*Helianthus annus*	Increase soil aggregation due to EPS production	(96)
P.entomophila *P. stutzeri* *P. syrnigae* *P. monteilli*	*Zea mays*	Increase Antioxidant enzyme, increase level of proline, sugar & other Osmolytes	(97)
Azospirillium lipoferum AZ1 *A.lipoferum* AZ9 *A. lipoferum* AZ45	*Triticum aestivum*	Increase relative water content (RWC) & decrease leaf water potential.	(98)

Pseudomonas florescens Pf1 *Bacillus subtilis* EPB5, EPB22 & EPB31	*Vigna radiata*	Increase root & shoot length, increase antioxidant enzymes like CAT, POX Accmulation of proline	(99)
B. amyloliquefaciens H40-B17 *B. licheniformis* H4TAPB18 *B. thuringenesis* H4DGRFB19 *Paenibacillus flavisporous* BKB30 *B.subtilis* RMPB44	*Zea mays*	Increase plant biomass, Increase RWC	(100)
Bacillus cereus AR156 *B. subtilis* SM21 *Serratia* spp. X421	*Cucumis sativa*	Decrease in leaf monodehydroascorbate content, decrease expression of drought triggered genes cAPX, rbcL, rbcS and increase in chlorophyll content, increase proline accumulation	(101)
Variovorax paradoxus	*Pisum sativum*	Increase nutrient uptake, ACCd decrease root ABA	(102)
Achromobacter xylosoxidans SF2 *Bacillus pumilis* SF3 &SF4	*Helianthus annus*	Increase RWC, Increase Salicylic acid in shoot	(103)
P. putida *P. florescens*	*Hyoscyamus niger*	Increase RWC, increase leaf no., increase antioxidant enzymes, proline accumulation	(104)
Bacillus pumilis DH11, *B. firmus* 40	*Solanum tuberosum*	Increase APX, SOD CAT, Increase ROS scavenzing enzyme	(105)
B. amyliliquefaciens 5113 *Azospirillium brasilense* N040	*Triticum aestivum*	Increase RWC, increase fresh & dry weight, decrease ROS	(106)
Azospirillum lipoferum GQ255950	*Zea mays*	Increase RWC, accumulation of free amino acid &proline	(107)
PGPR isolate 1 K, 9K & KB	*Zea mays*	Increase root and shoot length, increase RWC	(108)
Bacillus spp. KB122, KB129,	*Sorghum bicolor*	Increase shoot length, RWC, sugar,	(109)

KB133, KB142		chlorophyll, proline content	
Bacillus thuringiensis	*Triticum aestivum*	Increased activity of glutathione reductase (GR),CAT, superoxide dismutase (SOD), Alginate, IAA, Reduced emissions of stress volatiles	(110)
Pseudomonas aeruginosa	*Vigna radiata*	IAA, Up regulation of DREB2A, CAT1, DHN, Increased activity of SOD, POX, CAT	(111))
Pseudomonas spp.	*Zea mays*	EPS increased soil aggregation and water uptake	(112)
Acinetobacter, Pseudomonas	*Vitis vinifera*	IAA, EPS, Siderophore production	(85)
Bacillus thuringiensis	*Lavandula*	Decrease of GR, APX, Increased K+ content, Micronutrient uptake	(57)
Azospirillum brasilense	*Arabidopsis thaliana*	High ABA, Proline	(64)
Bacillus thuringiensis	*Trifolium repens*	Proline, APX activity decreased	(81)

4. Molecular advances to study drought responsive genes

Gene expression studies are a prominent tool to identify and evaluate the all-inclusive reaction of an organism to the environment (113). The transcriptome comprises the complete mRNA, present in a cell at any particular stage or under any environmental conditions. Different techniques for accessing the transcriptome are hybridization-based microarrays, RNA sequencing (114). Whole transcriptome sequencing of a potato colonizing strain i.e. *Burkholderia phytofirmans* PsJN was also made to examine its effect on drought tolerance (115). The study of rapeseed and its symbiote, *Stenotrophomonas rhizophila* through transcriptomic analysis helped in the recognition of spermidine, a growth regulator, formed under abiotic stress (116). Proteomics study under drought stress is useful in identifying target proteins and systems can be supported by a relative study of stress, non-stressed, and PGPR-primed plants (117). The increased expression of *bab18* (encoding late embryogenesis abundant protein), and *erd15* (encoding early response to dehydration 15), in *Paenibacillus polymyxa* B2 primed *Arabidopsis,* leading to improved drought tolerance (118). The proteomic studies of *Piriformospora indica* primed barley during drought demonstrated increased in antioxidants and photosynthetic proteins (119). To comprehend the system formed by nexus of numerous metabolic networks taking place in an ecosystem, metaproteomics is gaining popularity. Change in

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environmental conditions induces metabolism variance which leads towards the alteration exudation pattern (120). It has been well-documented that under the drought stress, dehydrin-like genes in sunflower (*Helianthus annuus*) roots and leaves are differentially expressed using RNA sequencing (121). Further, using 2-D polyacrylamide gel electrophoresis (2D-PAGE) some unique proteins responding towards drought has also been recognized in roots of wheat plants(122). In a combinatorial study, utilizing 2D-PAGE and differential display polymerase chain reaction (DD-PCR), six diffrentially expressed stress proteins were discovered under drought, in pepper plants primed with *Bacillus licheniformis* K11 (73). Using Real-Time PCR (RT-PCR), modulated hormonal levels and altered translational profile of a regulatory component (CTR1) of ethylene signaling pathway and DREB2 transcription factor were detected in *Bacillus subtilis* (LDR2) treated wheat plants during drought (123)**.** Using microarray analysis, the expression profile of numerous drought signaling response genes was reduced in the *Pseudomonas chlororaphis* O6-colonized *Arabidopsis thaliana*. mRNAs of the jasmonic acid-marker genes, VSP1 and pdf-1.2, salicylic acid regulated gene, PR-1, and the ethylene-response gene, HEL, were overexpressed in inoculated plants (79). For drought response approaches, microarray technique has been employed in plant-microbe interaction in modern wheat and wild emmer (124). Using Illumina sequencing (HiSeq 2000system) it was established that the participation of N_2 fixing bacteria *Gluconacetobacter diazotrophicus* PAL5 and sugarcane cv. SP70-1143 led to up-regulation of the ABA-dependent signaling genes imparting drought resistance in sugar cane cv. SP70-1143 (125). Thus, with the involvement of various high-end techniques, further new pathways could be discovered from rhizospheric inhabitants for their appropriate utilization, during drought.

5. Seed-coated bioinoculants in drought stress mitigation

The preparation of bio-formulations for plant growth promotion, soil fertility, and the destruction of plant pathogens provide environment-friendly substitute to classical agricultural chemicals (126). Yet, their extensive use is limited by the huge quantity of bio-inoculum required per plant (127). Additionally, lesser research on efficient inoculation procedures in large-acre crops further slow down their commercial level utilization. Bio-inoculants can directly be applied on soil (direct soil application), the seed (seed-applied inoculant), or on the whole plant (e.g., root dipping and foliar spray) (128). A recent study of simultaneous application of biochar and PGPR has reported an increase in RWC and essential nutrient uptake (129). Direct utilization of bio-inoculants on soil is beneficial when there are chances of seed damage by bio-inoculant, but is a cost-intensive method as a higher amount is required per land area

(130). Root dipping and foliar spray are commonly employed for inoculation of plants but the necessity of plant nursery formation and requirement of a higher amount of formulation, are its common drawbacks. Alternatively, seed-applied inoculants is an economical method and effectively places PGPR in its niche i.e. rhizosphere of desired crop (131). Seed coating is an effective technique for the generation of seed-applied inoculants (132). Microbial seed coating is done by combining binders, fillers, and inoculants along with seeds in basic mixing equipment (e.g., cement mixer) or more specialized machinery (e.g., fluidized bed). Binders are common adhesive compounds like Methylcellulose, CMC, Gum Arabic, polysaccharide PelGel, etc. Fillers are bulking agents like Peat, Talc, Lime, etc. Some compounds like alginate work as both filler and binder (133, 134). Recently, biochar and chitosan are also used for serving the dual purpose of filler and binders (135-137). Binders are applied to extend microbial survival. The right arrangement of the type and concentration of binder and filler is crucial for normal seed germination and further plant growth. A recent report using *Pseudomonas putida* coated chickpea seeds using starch and SiO_2 resulted in increased yield due to elevated biomass under drought (138). *Paenibacillus lentimorbus* B-30488 coated chickpea along with sodium alginate and calcium chloride ($CaCl_2$) improved the germination rate and colony-forming units (cfu) number, and resulted in better drought stress endurance (139). The report suggested the potent role of sodium alginate and $CaCl_2$ in stimulating the B-30488 biofilm and the coping against drought. Superabsorbent polymers (SAPs) are water -soluble polymeric compounds that are capable of withstanding over 100 times their weight in water and were used in agriculture since earlier times (140). SAPs are mixed with additive material in seed coating technologies to create encrusted or pelleted seeds (141, 142). By enhancing availability of water near the sown seed, SAP and hydro-absorbers, increased germination potential (143). Despite the plethora of published studies on the potential of various microbial inoculants to productivity enhancement and resistance against various biotic and abiotic stresses, only a limited portion has been expanded to mass-scale agricultural use. The common limiting factors for mass-production of bio-inoculants include failure of growth enhancement by lab-selected PGPR strains on the field (144, 145), the short expiry period of the formulation, destruction of microbial ecology by fumigants , and mineral-based pesticides and fertilizers. These challenges are to be overcome for allowing the widespread use of bio-inoculants sustainable agricultural systems.

6. Discussion

Drought is a significant limiting factor in agricultural productivity. This review provides a detailed study of drought effects on crop plants and encourages the implementation of plant growth-promoting rhizobacteria as an environment-friendly approach for tackling drought and leading to agricultural sustainability. Further, using molecular and omics-based techniques for the study of several drought-responsive genes has broadened our knowledge of diverse functional attributes of rhizobacteria under drought. For more ecologically responsible and sustainable agricultural activities, and cleaner & better food choices, microbial inoculants are the best potential candidates. Recent research indicates that rhizobacteria can impart drought tolerance in plants. Rhizobacteria-primed plants employ multifarious means including, osmotic adjustment, increased antioxidant level, phytohormone production, increased nutrient uptake, EPS production, releasing VOCs, etc confer resistance against desiccation. All these above mentioned mechanisms almost invariably function by improving RWC of the plant under drought. While it is well-established that PGPR treatment is associated with increased RWC, the exact mechanism behind this shift is unclear. PGPR-driven ABA surge in plants, followed by quick stomatal closure is widely believed to increase RWC under drought (146).However, another accepted viewpoint relates the spike in RWC with the changes in responsiveness of physiological functions including stomatal closure(147). Such divergent opinions highlight the importance of deciphering the mechanisms underlying bacterial-mediated drought tolerance through increased RWC. Moreover, designing PGPR-based bio-inoculants to assist plants in dealing with drought -stress is a new avenue in microbial use in dry land agriculture. Seed coating could be an efficient approach for rhizobacteria-based bio-inoculants since it effectively translocates the PGPR into the plant’s rhizosphere. However, very fewer studies focused on the rhizobacterial inoculants and their effectiveness under drought has been made so far. This review provides a possible sequence of steps for increasing the success rate of PGPR based bioinoculants (Figure 2). Using the present clues, a more focused study is required about determining the appropriate rhizobacterial strains, their effective delivery mechanisms, and field testing of potential candidates.

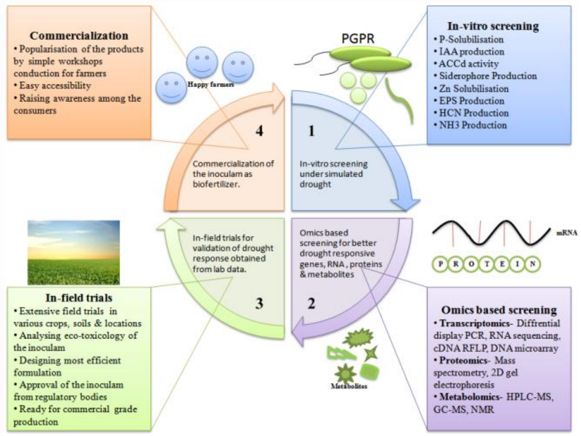

Fig 2- A possible sequence of steps for increasing the success rate of biofertilizers **(Step 1**- in-vitro screening of the PGPR isolates under simulated drought conditions; **step 2**- omics-based screening of strains showing better drought response; **step 3**- extensive field trials for validation of lab generated data to design effective bio-fertilizer and **step 4**- commercialization of the bio-inoculants)

Declarations

Ethics approval and consent to participate- Not applicable

Consent for publication- Not applicable

Availability of data and materials- Not applicable

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