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REVIEW

Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture

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Abstract Ethylene is a gaseous plant growth hormone produced endogenously by almost all plants. It is also produced in soil through a variety of biotic and abiotic mechanisms, and plays a key role in inducing multifarious physiological changes in plants at molecular level. Apart from being a plant growth regulator, ethylene has also been established as a stress hormone. Under stress conditions like those generated by salinity, drought, waterlogging, heavy metals and pathogenicity, the endogenous production of ethylene is accelerated substantially which adversely affects the root growth and consequently the growth of the plant as a whole. Certain plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia. Inoculation with PGPR containing ACC deaminase activity could be helpful in sustaining plant growth and development under stress conditions by reducing stress-induced ethylene production. Lately, efforts have been made to introduce ACC deaminase genes into plants to regulate ethylene level in the plants for optimum growth, particularly under stressed conditions. In this review, the primary focus is on giving account of all aspects of PGPR containing ACC deaminase regarding alleviation of impact of both biotic and abiotic stresses onto plants and of recent trends in terms of introduction of ACC deaminase genes into plant and microbial species.

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Introduction

Plant growth promoting rhizobacteria (PGPR) are beneficial soil bacteria, which may facilitate plant growth and development both directly and indirectly [50]. Direct stimulation may include providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate, while indirect stimulation of plant growth includes preventing phytopathogens (biocontrol) and thus, promote plant growth and development [47]. PGPR perform some of these functions through specific enzymes, which provoke physiological changes in plants at molecular level. Among these enzymes, bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase plays a well-understood role in the regulation of a plant hormone, ethylene and thus, growth and development of plants are modified [10, 48]. Bacterial strains containing ACC deaminase can, in part, at least alleviate the stress-induced ethylene-mediated negative impact on plants [48, 49, 120].

ACC deaminase has been widely reported in numerous microbial species of gram negative bacteria [11, 142], gram positive bacteria [16, 46], rhizobia [83, 139], endophytes [105, 124] and fungi [66, 93]. It is extensively studied in numerous species of plant growth promoting bacteria like *Agrobacterium genomovars* and *Azospirillum lipoferum* [19], *Alcaligenes* and *Bacillus* [16], *Burkholderia* [19, 105, 124], *Enterobacter* [107], *Methylobacterium fujisawaense* [84], *Pseudomonas* [16, 19, 61], *Ralstonia solanacearum* [19], *Rhizobium* [83, 139], *Rhodococcus*

[133], and *Sinorhizobium meliloti* [14] and *Variovorax paradoxus* [16].

The ACC deaminase metabolizes the root's ACC into α-ketobutyrate and ammonia and checks the production of ethylene which otherwise inhibits plant growth through several mechanisms [59]. The plants treated with bacteria containing ACC-deaminase may have relatively extensive root growth due to less ethylene [25, 126] and can better resist various stresses [25, 120]. Very recently, utilization of PGPR containing ACC deaminase activity in promoting plant growth and development both under stress and normal conditions and genetic manipulation of cultivars with genes expressing this enzyme has attracted much attention among the scientists [15, 120, 123]. Emphasis would, therefore, be laid in the following sections of this review on recent developments in this extremely important area of biotechnology.

Historical perspective of ethylene in plant physiology

Ethylene co-ordinates and regulates plant growth and functions via several mechanisms. Initially, ethylene was known as a ripening hormone but later investigations revealed that it merits equal status with the other classes of plant hormones due to its diverse effects and effective role in plant growth and development [10]. The recognition of ethylene as a plant growth regulator originated from observations of premature shedding of leaves, geotropism of etiolated pea seedlings when exposed to illuminating gas, early flowering of pineapples treated with smoke and ripening of oranges exposed to gas from kerosene combustion [1, 10]. Neljubow [98] was credited to have been the first to demonstrate the involvement of ethylene in the tropistic responses of plants. But later, intensive studies coupled with the advent of highly sophisticated analytical techniques like gas chromatography further elaborated its role in plant growth and development. Ethylene-induced physiological indicators of plant growth include release of dormancy, shoot and root growth differentiation, adventitious root formation, leaf and fruit abscission, induction of flowering and increased femaleness in dioecious plants, flower and leaf senescence, and fruit ripening [1, 68, 90]. Apart from all these, overproduction of ethylene leads to abnormal root growth, which imparts a visible dent on plant growth and development. Ethylene production in plant roots is accelerated in response to both biotic and abiotic processes [1, 5–10, 45]. As higher concentrations of ethylene have inhibitory effects on root growth and may lead to abnormal growth of the plants, it is imperative to regulate the ethylene production in the close vicinity of plant roots for normal growth and development of the plants.

Enzymes involved in regulation of ethylene level in plants

The knowledge of biosynthetic pathways of ethylene production in plants has made it possible for the plant physiologists to elucidate the mechanisms by which plants regulate the endogenous ethylene level for their normal growth and development. Mainly, the enzymes that degrade S-adenosylmethionine (derived from L-methionine) or ACC, have been shown to effectively reduce ethylene levels without drastically altering the physiology of the plant [62, 116, 117]. In this context, a number of enzymes have been investigated which help in lowering ethylene levels in the plants. Among these, the enzymes S-adenosylmethionine (SAM) hydrolase and SAM decarboxylase have been investigated to a lesser extent in relation to regulation of ethylene in plants [44, 75] while ACC synthase and oxidase have been studied extensively in numerous plant species [56, 76, 119, 127].

ACC-deaminase and its biochemistry

A pyridoxal 5-phosphate (PLP)-dependent polymeric enzyme, ACC deaminase was first studied in a soil bacterium *Pseudomonas* sp. strain ACP that degrades a cyclopropanoid amino acid, ACC to -ketobutyrate and ammonia [59]. Karthikeyan et al. [69, 70] have comprehensively described a scheme of structure for the ACC deaminase while providing an insight into the mechanism of unique pyridoxal-5-phosphate dependent cyclopropane ring-opening reactions of this enzyme in *Pseudomonas* sp. Recently, Hontzeas et al. [60] has thoroughly reviewed the reaction mechanisms involved in the functioning of ACC deaminase. Apart from all these, the biochemical and physical aspects of ACC deaminase have been investigated extensively by numerous researchers and summarized in Table 1. Glick [48] reported that there is a wide range (>100-fold) in the level of ACC deaminase activity among different organisms and those organisms that express high ACC deaminase typically bind non-specifically to a variety of plant surfaces. This group includes most, if not all, rhizosphere and phyllosphere organisms as well as endophytes, all of which can act as a sink for ACC produced as a consequence of plant stress. Moreover, these organisms display little preference for one particular plant over another [48]. On the other hand, low deaminase-expressing organisms bind only to specific plants or are expressed only in certain tissues, and they do not lower the overall level of ethylene in the plant, but rather prevent a localized rise in ethylene levels [48]. According to Glick [48] this group includes most, if not all, rhizobial as well as plants' ACC deaminases [48].

Table 1 Biochemical characterization of ACC deaminase from some selected microorganisms

Microorganism	*Pseudomonas* sp. strain ACP	*Hansenula saturnus*	*P. putida* GR12-2	*Penicillium citrinum*	*P. putida* UW4
Molecular mass (Da)	104–12,000	69,000	105,000	68,000	[a]
Sub unit molecular mass (Da)	36,500	40,000	35,000	41,000	41,800
Estimated number of subunits	3	2	3	2	[a]
Optimum pH	8.0–8.5	8.5	8.5	8.5	8.0
Optimum temperature (°C)	[a]	[a]	30	35	[a]
Km for ACC (mM)	1.5–9.2	2.6	[a]	4.6	3.4
Kcat (min^{-1})	290	[a]	[a]	[a]	146
Melting temperature (°C)	[a]	[a]	[a]	[a]	58–60
Reference	[58, 59]	[59, 93]	[64]	[66]	[61]

[a] Not known

Mode of action of bacterial ACC deaminase

The model description of the mode of action of PGPR containing ACC deaminase was precisely elaborated originally by Glick et al. [49]. They comprehensively addressed the question, how bacterial ACC deaminase having a low affinity for ACC, can effectively compete with the plant enzyme, ACC oxidase, which has a high affinity for the same substrate, with the result that the plant's endogenous ethylene concentration is reduced. They argued that the biological activity of PGPR relates to the relative amounts of ACC deaminase and ACC oxidase in the system under consideration [49]. For PGPR to be able to lower plant ethylene levels, the ACC deaminase level should be at least 100- to 1,000-fold greater than the ACC oxidase level. This is likely to be the case, provided that the expression of ACC oxidase has not been induced [49].

PGPR synthesize and secrete indole-3-acetic acid (IAA), which gets adsorbed on the seed or root surface of the plants [42, 57] from tryptophan and other small molecules present in seed or root exudates [145]. Some of the newly synthesized IAA is taken up by the plant and, in conjunction with the endogenous plant IAA can further stimulate plant cell proliferation and elongation. In the meanwhile, IAA stimulates the activity of the enzyme ACC synthetase to convert SAM into ACC [71]. According to the model outlined by Glick et al. [49], a significant portion of ACC may be exuded from plant roots or seeds and taken up by the soil microbes or hydrolyzed by the vital microbial enzyme ACC deaminase to yield ammonia and α-ketobutyrate. The uptake and subsequently hydrolysis of ACC by microbes decreases the amount of ACC outside the plant [49]. Furthermore, the equilibrium between the internal and the external ACC levels is maintained through exudation of more ACC into the rhizosphere. Soil microbial communities containing ACC deaminase activity cause plants to biosynthesize more ACC than the plant would otherwise need and stimulate ACC exudation from plant roots, while providing microorganisms with a unique source of nitrogen (ACC), and consequently, the growth of microorganism containing ACC deaminase is accelerated in the close vicinities of plant roots as compared to the other soil microorganisms [49]. By doing so, not only the ACC level is lowered within the plant but also the biosynthesis of the stress hormone ethylene is inhibited [49]. A schematic representation of this model is shown in Fig. 1. Thus, a plant inoculated with bacteria containing ACC deaminase exhibits

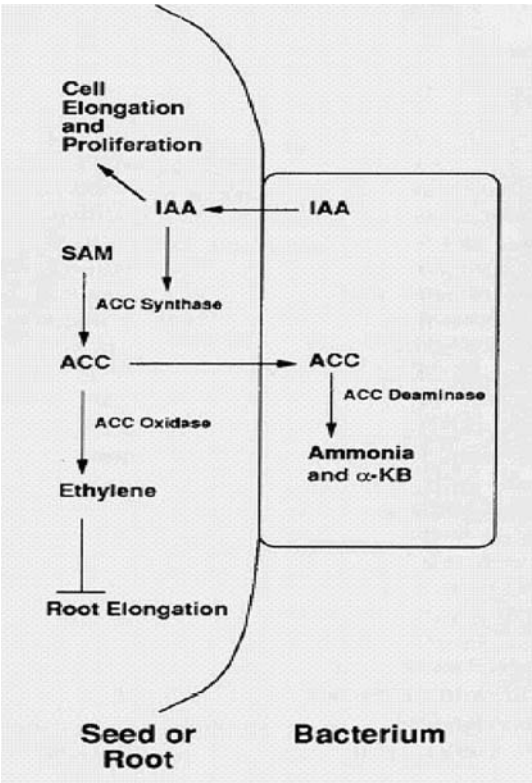

Fig. 1 Schematic representation of how a plant growth promoting rhizobacterium (PGPR) bound to either a seed or plant root lowers the ethylene concentration and thereby prevents ethylene inhibition of root elongation. *Source*: Ref. [49] with permission. *Key*: *IAA* Indole acetic acid, *ACC* 1-aminocyclopropane 1-carboxylic acid, *SAM* S-adenosylmethionine, *α-kB* α-ketobutyrate

more root growth. In a number of studies, inoculation with PGPR containing ACC deaminase has been unequivocally shown to alter the endogenous levels of ethylene, which subsequently leads to changes in plant growth. Some examples of plants inoculated with ACC deaminase containing bacteria and the physiological effects of the latter have been described in Table 2.

Chemical inhibitors of ethylene synthesis and bacterial ACC deaminase

Several different chemicals such as amino-ethoxyvinylglycine (AVG), aminooxyacetic acid (AOA) and 1-methylcyclopropene (1-MCP) have been successfully used to lower ethylene levels in plants or to alter a plant's sensitivity to ethylene especially during fruit ripening and flower wilting [1, 131]. In most cases these chemicals are expensive, less feasible or potentially harmful to the environment. On the other hand, use of PGPR containing ACC deaminase activity is more economical, environmental friendly and feasible in a natural soil and plant system. Moreover, the use of PGPR containing ACC deaminase activity is advantageous because ACC deaminase trait is common among a number of PGPR species, which are native to the rhizosphere and consequently possess a vast array of survival potential in the rhizosphere and rhizoplane. In addition, PGPR possess several other traits like synthesis of auxins, gibberellins, cytokines and/or polyamines, which directly promote plant growth [45, 106, 135, 149]. These characteristics make the selection of PGPR containing ACC deaminase more reliable than any other alternative.

Role of bacterial ACC deaminase in stress agriculture

The overproduction of ethylene in response to abiotic and biotic stresses leads to inhibition of root growth and consequently growth of the plant as a whole. Ethylene synthesis is stimulated by a variety of environmental factors/stresses, which hamper plant growth [1]. As described earlier, PGPR containing ACC deaminase regulate and lower the levels of ethylene by metabolizing ACC; a precursor of plant produced ethylene. These ACC deaminase PGPR boost plant growth particularly under stressed conditions by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses like salinity, drought, waterlogging, temperature, pathogenicity and contaminants. Applications of PGPR containing ACC deaminase in relation to the nature of stress are described below.

Salinity stress

Salinity stress boosts endogenous ethylene production in plants, which in most cases serves as a stress hormone [21, 32, 43, 101]. It is very likely that reducing salinity-induced ethylene by any mechanism could decrease the negative impact of salinity onto plant growth. Recent studies have revealed that plants inoculated with PGPR containing ACC

Table 2 Inoculation with PGPR containing ACC deaminase and subsequent physiological changes in plants

Plant species	PGPR	Comments	References
Brassica campestris	*Methylobacterium fujisawaense*	Bacterium promoted root elongation in canola.	[84]
Brassica campestris	*Bacillus circulans DUC1*, *Bacillus firmus DUC2*, *Bacillus globisporus DUC3*	Bacterial inoculation enhanced root and shoot elongation.	[46]
Brassica napus	*Alcaligenes* sp. *Bacillus pumilus* *Pseudomonas* sp. *Variovorax paradoxus*	Inoculated plant demonstrated more vigorous growth than the control (uninoculated).	[16]
Brassica napus	*Enterobacter cloacae*	A significant increase in the root and shoot lengths was observed.	[121]
Dianthus caryophyllus L.	*Azospirillum brasilense* Cd1843	Inoculated cuttings produced longest roots.	[79]
Glycine max	*Pseudomonas cepacia*	Rhizobacterium caused an early soybean growth.	[26]
Pisum sativum L.	*Rhizobium leguminosarum bv. viciae* 128C53K	Bacterium enhanced nodulation in plants.	[82]
Vigna radiata L.	*Pseudomonas* sp. *Bradyrhizobium* sp.	Bacterium promoted nodulation in mung bean.	[125]
Vigna radiata L.	*Pseudomonas putida*	The ethylene production was inhibited in inoculated cuttings.	[88]
Zea mays L.	*Enterobacter sakazakii* 8MR5 *Pseudomonas sp.* 4MKS8 *Klebsiella oxytoca* 10MKR7	Inoculation increased agronomic parameters of maize.	[11]
Zea mays L.	*Pseudomonas* sp.	Bacterium caused root elongation in maize.	[125]

deaminase were better able to thrive through the salinity stress while demonstrating a normal growth pattern. In this direction, Mayak et al. [86] reported that *Achromobacter piechaudii* having ACC deaminase activity significantly increased the fresh and dry weights of tomato seedlings grown in the presence of NaCl salt (up to 172 mM). The bacterium reduced the production of ethylene by tomato seedlings, which was otherwise stimulated when seedlings were challenged with increasing salt concentrations. However, the sodium content of the plant was not decreased whereas the uptake of phosphorous and potassium were slightly increased, which might have contributed in part, to the activation of processes involved in the alleviation of the adverse effect of salt on plant growth. The bacterium also increased the water use efficiency (WUE) in saline environment and helped in alleviating the salt suppression of photosynthesis. Recently Saravanakumar and Samiyappan [122] reported that *Pseudomonas fluorescens* strain TDK1 containg ACC deaminase activity enhanced the saline resistance in groundnut plants and increased yield as compared with that inoculated with *Pseudomonas* strains lacking ACC deaminase activity. Very recently, Cheng et al. [29] have also confirmed that ACC deaminase bacteria conferred salt tolerance onto plants by lowering the synthesis of salt-induced stress ethylene and promoted the growth of canola in saline environment. We too have observed almost similar results in the case of maize growth under salt stress in response to inoculation with ACC deaminase PGPR [95, 96].

Drought stress

Drought affects virtually all climatic regions of the world [146] and more than one-half of the earth is susceptible to drought every year [55, 74]. Drought is one of the major environmental stresses that limit the growth of plants and the production of crops. Plants respond to drought stress at cellular and molecular levels [23, 62, 129]. Like many other environmental factors, drought also induces accelerated ethylene production in plant tissues which leads to abnormal growth of a plant [85]. Mayak et al. [87] reported that ACC deaminase PGPR *Achromobacter piechaudii* ARV8 significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. In addition, the bacterium also reduced the production of ethylene by tomato seedlings exposed to water stress. During water scarcity, the bacterium did not influence the water content of plants; however, it significantly improved the recovery of plants when watering was resumed. Interestingly, inoculation of tomato plants with the bacterium resulted in continued plant growth both during water stress as well as when watering was resumed. Similarly, Dodd et al. [37] investigated the physiological responses of pea (*Pisum sativum* L.) to inoculation with ACC deaminase bacteria *Variovorax paradoxus* 5C-2 under moisture stress and watering conditions. The bacterial effects were more pronounced and more consistent under controlled soil drying (moisture stress conditions). In short term experiments, positive effects of ACC deaminase bacteria on root and shoots biomass, leaf area and plant transpiration was also observed. In case of long-term experiments, plants inoculated with ACC deaminase bacterium gave more seed yield (25–41%), seed number and seed nitrogen accumulation than uninoculated plants. Moreover, bacterial inoculation also restored nodulation in droughted pea plants to the levels of well-watered uninoculated plants. Very recently, we have also observed similar results. The inoculation with ACC deaminase bacteria partially eliminated the effects of water stress on growth, yield and ripening of *Pisum sativum* L. both in pot and field trials [3].

Waterlogging stress

Waterlogging enhances the biosynthesis of ethylene in roots and stem of plants. In flooding, ACC, which is synthesized in roots, is transported to plant shoots where it is converted to ethylene by ACC oxidase [22, 40]. The molecular basis for the increase in ethylene production observed in shoots of flooded tomato plants is due to an increase in the activity of both ACC synthase in the submerged roots and ACC oxidase in the shoots [27, 104]. The accelerated production of ethylene in the shoots of flooded tomato plants is responsible for the phenotype to demonstrate abnormal growth under flooding conditions [63]. Grichko and Glick [52] studied the effect of inoculation with ACC deaminase PGPR on tomato subjected to flooding. Seeds of wild-type tomato plants were inoculated either with *Pseudomonas putida* UW4, *Enterobacter cloacae* CAL2, *P. putida* (ATCC17399/pRKACC) or *P. putida* (ATCC17399/pRK415); the first three of these bacterial strains were carrying and expressing the gene for ACC deaminase. Tomato plants (55-day-old) were flooded for nine consecutive days before a number of physiological and biochemical parameters were recorded. Tomato plants inoculated with ACC deaminase PGPR showed substantial tolerance to flooding stress implying that bacterial ACC deaminase lowered the effects of stress induced ethylene.

Temperature stress

In nature, plants are sensitive to changes in temperature, and respond both to seasonal variations and more so to diurnal changes in the season. The heat stress in terms of so-called global warming is a serious threat to world agriculture [92, 115]. A fluctuation in temperature leads to hormonal imbalances in plants and thus their growth is

significantly affected [28]. Like many other abiotic and biotic factors, accelerated ethylene production under high and chilling temperatures has widely been reported by researchers both in plant tissues and microbial species in the rhizosphere [134, 143]. Plants with ACC deaminase expression may cope with this unfavorable situation by lowering ethylene level like that under other environmental stresses. Bensalim et al. [18] revealed that a plant growth promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN helped potato plants in maintaining normal growth under heat stress. Recently, Barka et al. [12] reported ACC deaminase activity in vitro and inoculation of grapevine (*Vitis vinifera* L.) cv. Chardonnay explants with the same bacterium (*Burkholderia phytofirmans* strain PsJN) enhanced plant growth and physiological activity at both ambient (26 °C) and low (4 °C) temperatures. Inoculation also increased root growth (11.8- and 10.7-fold increases at 26 and 4 °C, respectively) and plantlet biomass (6- and 2.2-fold increases at 26 and 4 °C, respectively). Moreover, the bacterium also significantly improved cold tolerance of plantlet compared to that of the nonbacterized control, which was more sensitive to exposure to low temperatures. Very recently, Cheng et al. [29] has also reported that a psychrotolerant ACC deaminase bacterium *P. putida* UW4 promoted canola plant growth at low temperature under salt stress. These few studies clearly demonstrated the potential of ACC deaminase in normalizing plant growth exposed to temperature extremes by lowering the accelerated ethylene induced by temperature stress.

Pathogenicity stress

Pathogenic microorganisms are a major and serious threat to food production and ecosystem stability worldwide. There has been a large body of literature describing potential uses of plant associated bacteria as agents for stimulating plant growth and conducive to soil and plant health [35, 36, 65, 137]. The widely recognized mechanisms of bio-control mediated by PGPR are competition for an ecological niche or a substrate, producing inhibitory allelochemicals, and inducing systemic resistance (ISR) in host plants to a broad spectrum of pathogens [20, 30, 80, 141].

Mostly, plant ethylene synthesis is enhanced with severity of pathogenic infection. In the meanwhile, some ethylene synthesis inhibitors are known to decrease the severity of pathogen infections in plants significantly [13, 39]. There are several reports, which support the hypothesis that ACC deaminase rhizobacteria have antagonistic effects against microbial pathogens. Yuquan et al. [148] reported isolation of ACC deaminase bacteria that showed very strong antagonism against plant pathogen *Fusarium oxysporum* (http://www.wanfangdata.com.cn/qikan/periodical.articles/shjtdxxb/shjt99/shjt9902/990223.htm (Accessed at June 11, 2007). To determine the potential use of PGPR containing ACC deaminase in biological control of various diseases, Wang et al. [142] conducted a series of experiments. Two biocontrol bacterial strains were genetically modified with the *P. putida* UW4 ACC deaminase gene to determine the effect of the transformed and non-transformed bacteria on a cucumbers disease caused by *Pythium ultimum*. The results of this study revealed that ACC deaminase containing bacterial strains were more effective in biocontrol than those without this enzyme. Similarly Donate-Correa et al. [38] have also reported the positive effect of ACC deaminase bacterium *Pseudomonas fluorescens* on *Chamaecytisus proliferus* (tagasaste) in antagonizing the growth of *Fusarium oxysporum* and *Fusarium proliferatum* in growth medium. Pandy et al. [105] reported that an ACC deaminase containing endophyte belonging to *Burkholderia* sp. exhibited antagonistic activity against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Contrarily, Rasche et al. [109] found no antagonistic effect of ACC deaminase bacteria against bacterial pathogen *Erwinia carotovora* sp. *atrospetica* (Eca). Interestingly, in another study, Rasche et al. [110] reported that ACC deaminase bacteria were also capable of antagonizing at least one of the two potato pathogens *Ralstonia solanacearum* and *Rhizoctonia solani*. It is also very likely that ACC deaminase bacteria, apart from directly antagonizing pathogens, support the plant's resistance against pathogen attack. They suggested that further research is needed to disclose the relationship between bacterial ACC deaminase and pathogenic antagonism. Moreover, it is very likely that ACC deaminase genes may also be present among a vast variety of pathogenic bacteria. Recently many researchers have reported ACC deaminase activity in plant pathogenic bacteria [19, 67, 130]. However, it is not clear that the presence of ACC deaminase in pathogenic bacteria could mask the pathogenic effects of these bacteria and promote plant growth. Very recently, a novel work was conducted by Belimov et al. [17] who concluded that bacterial ACC deaminase of *Pseudomonas brassicacearum* Am3 (pathogenic bacteria) can promote growth in tomato by masking the phytopathogenic properties of this bacterium only at lower concentration (inoculum size). Such evidences may imply that bacterial ACC deaminase could play a potential role in inducing disease tolerance in plants, however intensive future research work is needed for further understanding of this mechanism.

Contaminants stress

Heavy metals stress

Some but not all metals are essential or beneficial micronutrients required by plants for growth and development.

However, when present in excess, they may act as toxicants and suppress the plants growth [41]. In addition to this, high metal concentrations in the soil have also been shown to cause increased ethylene production [118, 120] and inhibition of root and shoot development, to reduce CO_2 fixation and limit sugar translocation [108]. Arshad et al. [4] have critically reviewed the application of PGPR containing ACC deaminase activity in phytoremediation of heavy metal contaminated soil environment. Some selected examples of the improved plant growth due to inoculation with ACC deaminase rhizobacteria under heavy metal stress are summarized in Table 3.

Organic contaminants stress

Organic pollutants in the soil environment, if present above permissible limits, hinder plant growth via several mechanisms including abnormal growth of affected plant species [2]. This abnormal growth of the plant root system might be partially due to accelerated ethylene production in plants grown in polluted soil environment. A few studies have revealed an accelerated production of ethylene in soil and plants treated with organic contaminants [31, 33, 63]. Recently, Reed and Glick [113] have studied the growth of canola (*Brassica napus*) seeds treated with PGPR in copper-contaminated and creosote-contaminated soil. In creosote-contaminated soils, the native bacterium was the least effective, and the transformed encapsulated ACC deaminase bacterium was the most effective in growth promotion. Arshad et al. [4] have recently reviewed the significance of PGPR containing ACC deaminase activity in improving the growth of plants in the presence of organic contaminants.

Air pollutants stress

The anthropogenic emission of CO_2, CO, SO_2 (sulfur dioxide), NOx, CH_4, and O_3 is playing havoc with agricultural and other ornamental plants in the close vicinities of industrial colonies. Air pollution, in addition to damaging plants, inhibits many enzyme systems and metabolic processes of plants [89]. Increased ethylene evolution by plants exposed to various environmental stresses i.e. air contaminants has been well documented [94, 138, 140, 144] and this hormone is now considered a major regulator of plant defense reactions, including cell death, in response to pathogen attack and air contaminant stresses, i.e. O_3 exposure. Many researchers reported that the inhibition of ethylene biosynthesis resulted in a significant reduction of O_3-induced leaf lesion formation [91, 94]. In this direction, the role of ACC deaminase in alleviation of air contaminants stresses has not been studied. It is very likely that PGPR can be utilized as a gene source for genetic modification of plants expressing the enzyme ACC deaminase against plant damage by air pollutants.

Wilting of flowers

The wilting of ornamental flowers caused by ethylene production is a major impediment in the success of flowering business. Ethylene production in flowers drastically decreases their shelf life. Ethylene and its precursor ACC have been shown to have a potential role in senescence and wilting of flowering species [114, 147]. An important characteristic of PGPR containing ACC deaminase activity has been shown to be the enhancement of shelf life of flowers incubated in suspension form [97]. On a commercial scale, shelf life of flowers could be increased to manifold by treating them with suspensions of PGPR containing ACC deaminase activity, which portends great prospects for the application of this biotechnological approach to commercial floriculture.

Nodulation

During the process of nodulation, the infection of roots with microsymbiont imposes biotic stress and results in increased ACC level in the infected roots. It has been well established that ethylene and its precursor ACC are the negative regulator of nodulation in numerous plant species [77, 100, 103]. The latest evidence has demonstrated that PGPR containing ACC deaminase activity promotes nodulation in legumes through inhibition of ethylene biosynthesis and consequently, they enhance symbiosis and nitrogen fixation in plants [102]. Cattelan et al. [26] reported that ACC deaminase rhizobacteria caused early growth and promoted nodulation in soybean (*Glycine max* (L.) Merr). Ma et al. [82] found that ACC deaminase rhizobacterium *Rhizobium leguminosarum* bv. *viciae* 128C53K enhanced the nodulation in *Pisum sativum* L. cv. Sparkle by modulating ethylene levels in the plant roots during the early stages of nodule development. Contrarily, inoculation with ACC deaminase minus mutants strains resulted in decreased nodulation compared with ACC deaminase bacteria. Dey et al. [34] reported increased number of nodules in peanut (*Arachis hypogaea* L.) plants treated with *Pseudomonas fluorescens* exhibiting ACC deaminase activity. In a field study, inoculation of *Arachis hypogaea* L. plants with ACC deaminase bacterium *Pseudomonas fluorescens* enhanced nodule number and dry weight.. Uchiumi et al. [139] reported that an up-regulated gene in bacteroids, mlr5932, and encoding ACC deaminase activity was involved in enhanced nodulation in *Lotus japonicus* plants. Pandey et al. [105] isolated an endophytic ACC deaminase bacterium capable of modulating nodulation in *Mimosa pudica.* We have also found that co-inoculation with *Bradyrhizobium* plus ACC deaminase rhizobacteria increased nodulation in mung bean compared to inoculation with *Bradyrhizobium* only [125].

Table 3 Alleviation of impact of heavy metal stresses on plants by PGPR containing ACC deaminase

Plant species	PGPR containing ACC-deaminase	Contaminant	References
Brassica napus	*Kluyvera ascorbata* SUD165	Plant demonstrated normal growth under high levels of Ni^{2+}, Pb^{2+}, Zn^{2+}, and CrO_4^{2-}.	[24]
Brassica juncea L.	*Kluyvera ascorbata* SUD165 *Kluyvera ascorbata* SUD165/26	Toxic effects of heavy metals (Ni^{2+}, Pb^{2+}, and Zn^{2+}) were not pronounced in inoculated plants.	[25]
Brassica juncea L.	*Variovorax paradoxus*, *Rhodococcus* sp.	Plant growth was improved in Cd^{2+} supplemented media in response to inoculation.	[14]
Brassica juncea L.	*Pseudomonas brassicacearum*, *Pseudomonas marginalis*, *Pseudomonas oryzihabitans*, *Pseudomonas putida*, *Pseudomonas* sp., *Alcaligenes xylosoxidans*, *Alcaligenes* sp., *Variovorax paradoxus*, *Bacillus pumilus*, and *Rhodococcus* sp.	The bacteria were tolerant to Cd^{2+} toxicity and stimulated root elongation of rape seedlings in the presence of 300 μM $CdCl_2$ in the nutrient solution.	[16]
Lycopersicum esculentum Mill.	*Kluyvera ascorbata* SUD165 *Kluyvera ascorbata* SUD165/26	Toxic effects of the heavy metals (Ni^{2+}, Pb^{2+}, and Zn^{2+}) were not pronounced in inoculated plants.	[25]
Phragmites australis	*Pseudomonas asplenii* AC[a]	Inoculation resulted in normal plant growth under high levels of Cu^{2+} and creosote.	[112]
Pisum sativum L.	*Pseudomonas brassicacearum* Am3, *Pseudomonas marginalis* Dp1	Inoculation with bacteria counteracted the Cd-induced inhibition of nutrient uptake by roots.	[120]
Pisum sativum L.	*Pseudomonas brassicacearum*, *Pseudomonas marginalis*, *Pseudomonas oryzihabitans*, *Pseudomonas putida*, *Pseudomonas* sp., *Alcaligenes xylosoxidans*, *Alcaligenes* sp., *Variovorax paradoxus*, *Bacillus pumilus*, and *Rhodococcus* sp.	The bacteria were tolerant to Cd^{2+} toxicity and stimulated root elongation of rape seedlings in the presence of 300 μM $CdCl_2$ in the nutrient solution.	[16]

[a] Transgenic bacteria

Table 4 Transgenic plants with bacterial ACC-deaminase genes expression

Plant species	Source of ACC-deaminase gene	Promoter	Comments	References
Lycopersicum esculentum L.	*Pseudomonas* sp.	(CaMV) 35*S*	Fruit ripening was delayed substantially.	[72, 73]
Petunia hybrida, *Nicotiana tabacum*, *Lycopersicon esculentum*	*Pseudomonas chloroaphis*	[a]	Senescence of flowers was delayed. Similarly ripening was also delayed in tomato plants.	[73]
Lycopersicum esculentum L.	*Pseudomonas chloroaphis.*	(CaMV) 35*S*	Ripening time was extended.	[111]
Petunia hybrida	*Pseudomonas* sp.	[a]	Transgenic plants exhibited a significant reduction in ACC contents of pollen.	[78]
Lycopersicum esculentum L.	*Enterobacter cloacea*	(CaMV)35*S*, *rol*D and *prb*-1b	Plant demonstrated good growth under the stress of heavy metals like Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}.	[51]
Lycopersicum esculentum	Heinz 902 expressing the bacterial gene	(CaMV) 35*S*, *rol*D and *prb*-1b	Plants showed an increased tolerance against flooding stress.	[53]
Lycopersicum esculentum	[a]	(CaMV) 35*S*, *rol*D and *prb*-1b	The reduced ethylene synthesis resulted into an increased tolerance against disease.	[117]
Enterobacter cloacae CAL2	[a]	Double 35*S*	Transgenic plants showed more prolific growth.	[99]
Lycopersicon esculentum	*P. putida* UW4	*prb*-1b	Plants demonstrated relatively more vigorous growth than control (non-transgenic plants).	[136]
Lycopersicon esculentum Mill., *cv.* Heinz 902	[a]	(CaMV) 35*S*, *rol*D and *prb*-1b	The parameters like plant growth, leaf fluorescence, protein and chlorophyll content, fruit weight, and also lycopene and β-carotene contents in fruits were more in transgenic than in non-transgenic plants.	[54]
Brassica napus	The root specific *rolD* promoter from *Agrobacterium rhizogenes*	(CaMV) 35*S* and *rol*D	Plants showed a great tolerance toward Ni^{2+} toxicity.	[132]
Brassica napus	*Agrobacterium rhizogenes*	(CaMV) 35*S*, *rol*D and *prb*-1b	The salt tolerance in plants was increased manifold.	[123]

[a] Not known

Recent advances at molecular level

In the present scenario, agriculture sector is confronted to many stress(es) and calls for efforts towards biotechnological revolution (gene revolution) after green revolution. It is highly likely that biotech revolution may better address the long lasting challenges like drought, salinity, pest attack and pollution. Recently, efforts have been made to introduce specific genes into plants to enable them to cope with multifaceted environmental stresses. In the same direction, introduction of specific genes responsible for the expression of particular enzymes like ACC deaminase from microbial species directly into crop plants has received great attention in the last few decades. Some recent advances at molecular level have been described in the following sections.

Genetically engineered bacteria expressing ACC deaminase genes

Genetic manipulation of ACC deaminase trait in bacteria has not been much attempted. This is most likely due to (1) ACC deaminase trait is widely found among soil indigenous microbial species, (2) survival and functioning of wild type microbial species containing ACC deaminase is better than genetically engineered microorganism expressing ACC deaminase genes and/or (3) degree of success in transforming the ACC deaminase in plants has been quite successful [4, 10]. However, the genetically modified bacteria could be useful for developing better understanding of mechanisms responsible for induction of tolerance in plants inoculated with ACC deaminase bacteria against both biotic and abiotic stresses [47, 128]. Recent studies have also demonstrated that genetic modification of PGPR expressing ACC deaminase genes helped in modulation of nodulation in legumes and biological control of plant disease [81, 142].

Genetically engineered plants expressing ACC deaminase activity

The plant growth promotion observed in response to inoculation with bacteria containing ACC deaminase provoked scientists to develop transgenic plants with the expression of ACC deaminase genes [10]. Of late, an extensive work has been going on exploiting the potential of ACC deaminase genes in regulation of ethylene level in plants exposed to various kinds of stresses like salinity, drought, waterlogging, pathogenicity, heavy metals, etc. alongwith its potential role in phytoremediation of contaminated soil environment. During last decade, an intensive work has been conducted on this aspect. Some recent examples of transformed plants expressing ACC deaminase and subsequently changes in their physiological responses have been summarized in Table 4.

Conclusion

The present day agriculture is confronted with a hydra-headed stress induced by both biotic and abiotic factors. The various kinds of stresses discussed in this review accentuate the biosynthesis of ethylene, which in most cases inhibits plant growth through several mechanisms at molecular level. In the present scenario, the application of PGPR containing ACC deaminase is vital to regulate the plant ethylene. However, some beneficial aspects of PGPR, i.e. their role in salinity, drought, waterlogging, biocontrol, temperature and nutritional stresses and in cut-flowers industry and nodulation in legumes have not been thoroughly exploited. On a commercial scale, application of PGPR containing ACC deaminase in agriculture might prove beneficial and could be a sound step towards sustainable crop production and conservation. While genetic modification of all plant species is however not possible due to many handicaps i.e. proprietary rights and international trade agreements on genetically modified (GM) crops and limitations in DNA recombinant technology in some regions of the world, the use of PGPR containing ACC deaminase activity alongwith other innovations could prove to be a cost effective and environment friendly strategy to ensure sustainable agriculture.

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