

# A Review on Abiotic Stress Tolerance and Plant Growth Metabolite Framework by Plant Growth-Promoting Bacteria for Sustainable Agriculture

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To overcome abiotic stress such as high salinity or drought, plants will synthesise specific metabolites to enhance the tolerance level. These metabolites are used as markers to relate to the potential metabolite pathways to alleviate salinity or drought stress with enhanced tolerance. The application of plant growth-promoting bacteria (PGPB) is of high potential in reducing the symptoms induced under salinity or drought stress. However, limited studies have reported on the potential metabolite markers to represent the relationship between PGPB and plants to alleviate the salinity or drought stress. This review aims to summarise and develop a novel metabolite framework to relate the potential metabolite markers synthesised under salinity or drought stress as induced by PGPB. The metabolite framework is built based on a range of PGPB-induced metabolite markers modulated in different plants for stress alleviation and growth enhancement. From this review, major metabolite markers induced by PGPB under salinity and drought stress were identified as amino acids (ethylene, indole-3-acetic acid, salicylic acid, and proline) and isoprenoid (abscisic acid) in different plants. This framework is vital for constructing the metabolite network to decipher the underlying mechanisms for PGPB to enhance the tolerance of plants under salinity or drought stress in the future.

## 1. Introduction

A range of soil amendment methods has been implemented to promote sustainable soil management in agriculture practices (Lim et al., 2017). High salinity and drought are abiotic stresses that could compromise soil physicochemical properties and microbial functional diversity, causing retarded plant growth and reduced crop yield. Salinity stress is caused by the accumulation of different water-soluble salt ions in the soil, such as sodium, magnesium, and potassium. Drought affects the hydrological cycle in soil and causes water stress to the plants. The alleviation of these stresses in plants to promote plant growth is vital for sustainable agriculture practices. Plant growth-promoting bacteria (PGPB) are commonly used in agriculture due to its beneficial effects in promoting plant growth and helping plants to withstand high salinity and drought. PGPB, including rhizospheric bacteria, endophytic bacteria, symbiotic bacteria, and phyllospheric bacteria have been reported to promote plant growth under abiotic stresses (Glick, 2014). Forni et al. (2017) described the metabolic changes and altered hormonal activity, to ameliorate salinity and drought stresses, in plant added with PGPB. Numan et al. (2018) reported the role of PGPB in producing metabolites like indole-3-acetic acid (IAA) and cytokinins to enhance salinity tolerance in plants and promote plant growth. Goswami and Deka (2020) revealed a range of

bacterial phytohormones and metabolites, including IAA and abscisic acid (ABA) to relieve the abiotic stress of the plant.

PGPB has been reported to produce beneficial metabolites such as the IAA, a plant hormone of the auxin class (Belimov et al., 2015). The known precursor of auxin is the amino acid tryptophan. Auxin is linked with improved lateral roots and root hair formation (Overvoorde et al., 2010). The length and distribution of roots affect the ability of plants to uptake water and nutrients efficiently from the soil. Auxin-producing PGPB can inhabit the plant roots and enhance the tolerance of the plant against drought stress. PGPB also produce an enzyme, the amino acid 1-amino-cyclopropane-1-carboxylate (ACC) deaminase to restrict the production and accumulation of plant ethylene, and keeping them (ACC and ethylene) balanced under stress condition (Glick et al., 2014).

Plants adapt to high salinity or drought by producing antioxidants and accumulating compatible solutes, such as salicylic acid (SA) and proline. SA has been known to ameliorate salinity stress by enhancing plant productivity through regulating photosynthesis and producing antioxidative enzymes (Rivas-San Vicente and Plasencia, 2011). The modulation of SA biosynthesis during PGPB-plant interaction also helps to comfort stressed plant and promotes plant growth under drought and high salinity. PGPB could alter the level of proteinogenic amino acid proline in the plant (Tiwari et al., 2016), which is derived from the amino acid L-glutamate.

Previous studies have reported the possibility of using single or combinations of metabolites as markers to predict how PGPB affect plant performance under abiotic stress. However, those studies did not give clear insights in presenting the metabolite markers in PGPB-plant interaction to enhance the tolerance of plants under salinity or drought stress. More classes of metabolites such as amino acid, isoprenoid, carbohydrate, lipid and polyamine should be included to gain more insight of the PGPB-plant interaction in alleviating the stress, especially salinity or drought stress.

This paper reviews a multiple collective class of metabolite markers related to enhancing salinity or drought stress tolerance and plant growth as mediated by PGPB. A novel framework is constructed to illustrate the interrelations of more classes of metabolite markers to promote specific plant growth under these stresses. This framework serves as a powerful tool to further understand the underlying metabolites leading to specific plant growth-promoting properties, e.g. longer roots, improved seed germination, and higher chlorophyll content.

## 2. Methods

Data collection was conducted from the peer-reviewed international journals and web sources such as Google Scholar and ScienceDirect using the keywords like “PGPB”, “plant growth”, “metabolite”, and “abiotic stress”. In this review, the data collection only focused on studies related to salinity and drought stresses alleviation as mediated by PGPB. Selected studies were published within the year of 2010 to 2020. The data from the selected studies were tabulated and developed into a metabolite framework.

## 3. A metabolite framework for mapping plant tolerance markers under abiotic stress

PGPB have been reported to alleviate abiotic stresses by regulating the levels of essential primary and secondary metabolites. Table 1 shows the key metabolites reported following the addition of PGPB in the plant to alleviate the stress. Amino acids and isoprenoid metabolite are the two main classes of metabolites induced by PGPB to provide a range of plant-promoting effects. Specific amino acid derivatives (such as ethylene and its immediate precursor ACC, IAA, SA, and proline) and metabolite from isoprenoid pathways (ABA) play essential roles in signalling the adaptive stress responses and growth promotion under salinity and drought stresses as mediated by PGPB.

The alteration of ACC to a lower level by PGPB was evident to help plants adapt to drought stress while enhancing plant growth. Belimov et al. (2015) reported that the selected PGPB strains (Table 1) decreased rhizosphere ACC concentrations (decreased by about 30 to 55 % and 11 to 68 % in two different potato cultivars, when compared to control) around the potato roots due to PGPB enzyme ACC-deaminase activity.

The lower level of ACC mediated by PGPB was also reported by Zhang et al. (2018) in the leaf of wheat under drought condition. The degradation of ACC by bacterial ACC deaminase may be one of the reasons for the enhanced growth of plants by restricting excessive ethylene production in plants (Chen et al., 2013), which was stimulated under soil water deficit condition.

Yao et al. (2010) showed that under salinity stress, cotton seedlings treated with PGPB resulted in enhanced germination rate, increased number of healthy stands, and improved plant biomass. The selected PGPB strains helped the cotton plant to uptake ions and nutrients, along with the effects of other plant growth-promoting metabolites such as IAA and ABA. PGPB significantly increased the endogenous IAA concentration (by 51.63 % when compared to control) while decreasing the ABA concentration (by 23.25 % when compared to control) in the cotton seedlings, countering the opposite effect caused by salinity stress on both metabolites. The

metabolite data from Table 1 are classified for their role, either as growth promoter or stress indicator, for the enhanced tolerance in Table 2.

*Table 1: Summary of the key metabolites mediated by PGPB to enhance plant tolerance against salinity or drought stress*

| Stress, PGPB strains   | Plant, species                              | Key metabolites*   | Plant growth enhancement  | Reference             |
|--|---|--|---|-----------------------|
| <b>Salinity</b>  |   |  |   |                       |
| <i>Pseudomonas putida</i> Rs-198   | Cotton ( <i>Gossypium hirsutum</i> )        | Amino acid (IAA)<br>Isoprenoid (ABA)   | Increased germination rate, plant biomass   | Yao et al. (2010)     |
| <b>Drought</b>   |   |  |   |                       |
| <i>Phyllobacterium brassicacearum</i> STM196   | Thale cress ( <i>Arabidopsis thaliana</i> ) | Isoprenoid (ABA)   | Increased plant biomass, lowers transpiration and photosynthesis                                  | Bresson et al. (2013) |
| <i>Bacillus spp.</i> KB122, KB129, KB133, and KB142  | Sorghum ( <i>Sorghum bicolor</i> )          | Amino acid (proline)<br>Carbohydrate (SS)  | Increased plant biomass, chlorophyll content, relative water content, soil moisture content       | Grover et al. (2014)  |
| <i>Achromobacter xylosoxidans</i> Cm4, <i>Pseudomonas oryzihabitans</i> Ep4 and <i>Variovorax paradoxus</i> 5C-2 | Potato ( <i>Solanum tuberosum</i> )         | Amino acid (ethylene)  | Increased plant biomass   | Belimov et al. (2015) |
| <i>Pseudomonas putida</i> MTCC5279   | Chickpea ( <i>Cicer arietinum</i> )         | Amino acid (ethylene, proline, SA)<br>Lipids (MDA, jasmonate)<br>Carbohydrate (SS) | Improved seed germination, plant biomass and relative water content                               | Tiwari et al. (2016)  |
| <i>Enterobacter mori</i> AL, <i>Enterobacter asburiae</i> BL and <i>Enterobacter ludwigii</i> CL2                | Wheat ( <i>Triticum aestivum</i> )          | Amino acid (ethylene)  | Increased plant biomass   | Zhang et al. (2018)   |
| <i>Bacillus megaterium</i> BOFC15  | Thale cress ( <i>Arabidopsis thaliana</i> ) | Polyamine (spermidine, spermine)<br>Isoprenoid (ABA)<br>Lipids (MDA)               | Increased plant biomass, improved root system architecture, and increased photosynthetic capacity | Zhou et al. (2016)    |

\*MDA – malondialdehyde; SS – soluble sugars

*Table 2: The role of the metabolites in enhancing tolerance against salinity or drought stress mediated by PGPB*

| Metabolite classes involved | Salinity | Drought | Growth promoter | Stress indicator |
|-----------------------------|----------|---------|-----------------|------------------|
| Amino acid                  |          |         |                 |                  |
| IAA                         | ✓        |         | ✓               |                  |
| Proline                     |          | ✓       | ✓               |                  |
| Ethylene/ACC                |          | ✓       | ✓               |                  |
| SA                          |          | ✓       | ✓               |                  |
| Isoprenoid                  | ✓        | ✓       |                 | ✓                |
| Carbohydrate                |          | ✓       | ✓               |                  |
| Lipids                      |          | ✓       |                 | ✓                |
| MDA                         |          | ✓       |                 |                  |
| Jasmonate                   |          | ✓       | ✓               |                  |
| Polyamine                   |          | ✓       | ✓               |                  |
| Spermidine                  |          | ✓       | ✓               |                  |
| Spermine                    |          | ✓       | ✓               |                  |

Table 2 shows that relatively more metabolite classes have been analysed to relate drought tolerance as mediated by PGPB than for salinity tolerance. There are more research scopes to characterise the principal metabolites contributing to enhanced tolerance on salinity stress in the plant. Despite lacking IAA analysis for plant tolerance under drought stress (Table 2), the profiling of IAA should be included to investigate the effect of PGPB inoculation on the metabolite modulations comprehensively. IAA plays a crucial role in signalling processes between PGPB and plant for both the development of roots and shoots. A deeper root system with higher root densities is likely to be the mechanism for stress alleviation as it improves the extraction of soil water under drought stress conditions (Lopes et al., 2011). IAA is known as a plant growth hormone; ABA is an essential stress-inducible plant hormone and growth inhibitor, that is regarded as a physiological stress marker in plants.

A higher level of ABA was found in the *Arabidopsis* plants mediated by PGPB compared to the control under drought (Bresson et al., 2013), see Table 1. The PGPB-inoculated plant exhibits higher water retains due to higher root biomass. The plant experienced significantly lower water loss through transpiration, that may reflect a better drought resistance strategy. This enhanced tolerance was correlated with a PGPB-induced delay in the transition from plant vegetative to reproductive developmental stage, which causes higher biomass accumulation before the flowering time of the plant. Higher concentrations of ABA in the leaves might lead to lower transpiration rate and resulting in a reduced plant growth rate.

Zhou et al. (2016) demonstrated that the selected PGPB strain improved plant drought tolerance through alteration of cellular ABA levels, activated adaptive responses, and modification of cellular polyamine of plants represented by the increased free spermidine and spermine levels. They reported that the increase in ABA level of drought-stressed plants was associated with the increased polyamine level. The inoculated plants were able to scavenge reactive oxygen species (ROS) under drought condition, indicating that the PGPB was able to detoxify the cellular ROS and to mitigate oxidative injury to the cellular structure. This effect was further proven by the lower level of malondialdehyde (MDA) observation. MDA level is commonly known as a marker of oxidative stress and the antioxidant status in plants, and it is one of the most common by-products of lipid peroxidation during oxidative stress. Reduced level of ROS in the inoculated plants was due to the activation of enzymatic antioxidant defence systems. The spermidine secreted by PGPB possibly activated the enzymatic systems to eliminate ROS.

A similar finding was reported by Tiwari et al. (2016), where PGPB-inoculation assisted in reducing membrane damage by lowering MDA accumulation (38.6 % and 123 % decline in two different chickpea cultivars at 7<sup>th</sup> d drought stress, as compared to their individual inoculated plants), resulting in beneficial effects on chickpea seedlings by protecting them from membrane damage and oxidative stress under drought stress. The PGPB inoculation also increases proline and total soluble sugars (SS) in leaves with progression of drought stress. The gene expression profiling showed a low level of ethylene, a higher level of SA-responsive gene expression, and up-regulation of jasmonate signalling pathway gene in PGPB-inoculated plant under drought stress. A higher level of jasmonate content such as jasmonic acid in the plants can prevent oxidative stress damage and protects the plant.

A variation in proline and SS content have been reported in PGPB-inoculated flowering plant sorghum under different drought stress conditions (Grover et al., 2014). The results suggested that the modulation of proline and SS as the compatible solutes is vital to help in maintaining osmotic turgor. Proline also helps to alleviate drought-induced oxidative damages to cell systems in the plant.

The data, as reviewed above, are quite complex and difficult to follow. A metabolite framework could be drawn to decipher the inter-relations of the metabolite markers, including the plant-growth-promoting and stress indicator. Previous studies have investigated the effects of PGPB inoculation on specific plant growth performance under salinity and drought stress. Zhou et al. (2016) proposed a diagram to illustrate the physiochemical alterations for drought resistance in plants mediated by PGPB. Forni et al. (2017) presented an overview of salt- and drought-stress responses in plants with PGPB inoculation to promote stress tolerance. Goswami and Deka (2020) illustrated the effects of abiotic stresses on plants and the roles of PGPB in alleviating stress in soil. A comparison of metabolites either as growth-promoting or stress marker for salinity or drought stress has been limited.

A metabolite framework has been developed in this study to illustrate a comprehensive process where the effects of PGPB-induced metabolite markers are related to specific plant growth enhancement under salinity or drought stress, as shown in Figure 1. The framework provides a better insight into the metabolite markers and the potential relationships between the metabolite markers induced by PGPB concerning the specific plant growth enhancement. Each metabolite marker produced by PGPB has a specific function in alleviating stress and promoting specific plant growth. The metabolite framework, which shows the plant-PGPB interactions to relieve salinity or drought stress, facilitates future studies (e.g. kinetic studies) on the complex modulation of metabolites under abiotic stress.

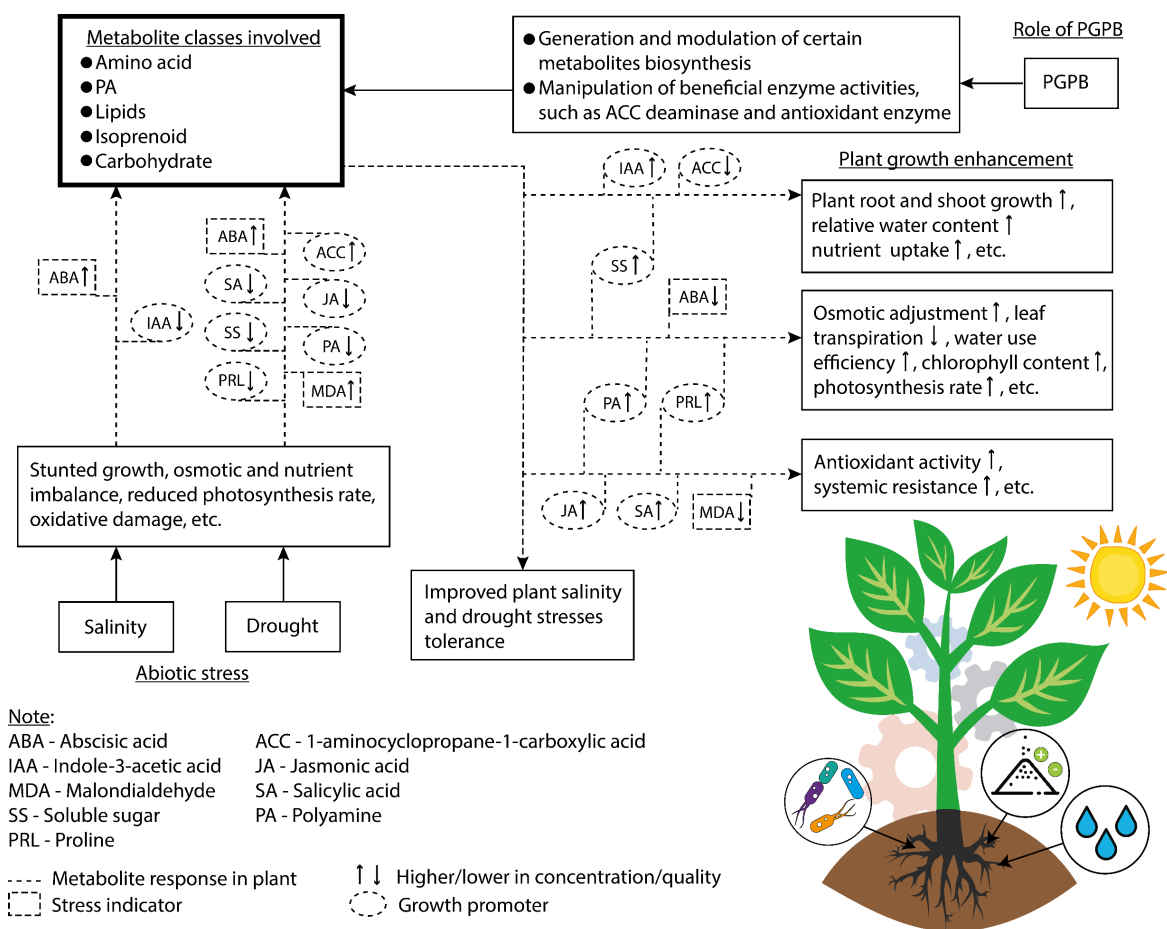


Figure 1: PGPB-plant interaction framework under salinity and drought stress conditions. These metabolite alterations and physiological changes lead to plant adaptation to stress

#### 4. Conclusion

Investigations on the significance of plant metabolite regulations in adverse stress conditions, such as high salinity or drought, has noticeably expanded. Salinity or drought stress tolerance in plants comprises of a very complicated process involving various metabolites to either serve as plant growth-promoting markers or stress markers. Based on different responses of PGPB-induced metabolites subjected to salinity or drought stress, the current review presented a novel metabolite framework model to illustrate further the role of PGPB-induced metabolite markers in plant and their inter-relations in alleviating salinity or drought stress, resulting in specific plant growth enhancement.

Under salinity and drought stresses, the key metabolites from the amino acid and isoprenoid metabolite classes are found to enhance stress tolerance in different plants. The modulation of these metabolites among others and alteration of beneficial enzyme activities are regarded as the reasons for improving the plant growth, such as enhanced seed germination, expanded and elongated root system leading to improved uptake of water and nutrients, and increased in chlorophyll content, all of which are inter-related to decipher the detailed mechanisms to relieve the abiotic stress. While amino acid and isoprenoid metabolite classes are more commonly analysed for salinity stress tolerance, other metabolite classes such as carbohydrate, lipids and polyamine are often included and profiled for drought tolerance. This study also add-on more classes of metabolites such as lipids and polyamines and further illustrate the inter-relations of the metabolite markers for salinity or drought stress; these inter-relations have not been reported before.

The key metabolites outlined shows the steady-state level of metabolites. A detailed kinetics and flux analyses of the metabolites will be necessary for a better insight of metabolite modulations in response to abiotic stresses as mediated by PGPB in future studies. The complex inter-relations, as shown by the novel metabolite framework, will help unravel the detailed mechanisms on how PGPB could alleviate abiotic stresses.

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## References

- Belimov A.A., Dodd I.C., Safronova V.I., Shaposhnikov A.I., Azarova T.S., Makarova N.M., Davies W.J., Tikhonovich I.A., 2015, Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*), *The Annals of Applied Biology*, 167(1), 11-25.
- Bresson J., Varoquaux F., Bontpart T., Touraine B., Vile D., 2013, The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*, *New Phytologist*, 200(2), 558–569.
- Chen L., Dodd I.C., Theobald J.C., Belimov A.A., Davies W.J., 2013, The rhizobacterium *Variovorax paradoxus* 5C-2, containing ACC deaminase, promotes growth and development of *Arabidopsis thaliana* via an ethylene-dependent pathway, *Journal of Experimental Botany*, 64, 1565–1573.
- Forni C., Duca D., Glick B.R., 2017, Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria, *Plant Soil*, 410, 335–356.
- Glick B.R., 2014, Bacteria with ACC deaminase can promote plant growth and help to feed the world, *Microbiological Research*, 169(1), 30–39.
- Goswami M., Deka S., 2020, Plant growth-promoting rhizobacteria-alleviators of abiotic stresses in soil: A review, *Pedosphere*, 30(1), 40–61.
- Grover M., Madhubala R., Ali S.Z., Yadav S.K., Venkateswarlu B., 2014, Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions, *Journal of Basic Microbiology*, 54(9), 951-961.
- Lim L.Y., Lee C.T., Lim J.S., Klemeš J.J., Ho C.S., Mansor N.N.A., 2017, Feedstock amendment for the production of quality compost for soil amendment and heavy metal immobilisation, *Chemical Engineering Transactions*, 56, 499-504.
- Lopes M.S., Araus J.L., van Heerden P.D., Foyer C.H., 2011, Enhancing drought tolerance in C<sub>4</sub> crops, *Journal of Experimental Botany*, 62(9), 3135-3153.
- Numan M., Bashir S., Khan Y., Mumtaz R., Shinwari Z.K., Khan A.L., Khan A., Al-Harrasi A., 2018, Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: A review, *Microbiological Research*, 209, 21–32.
- Overvoorde P., Fukaki H., Beeckman T., 2010, Auxin control of root development, *Cold Spring Harbor Perspectives in Biology*, 2(6), a001537.
- Rivas-San Vicente M., Plasencia J., 2011, Salicylic acid beyond defence: its role in plant growth and development, *Journal of Experimental Botany*, 62(10), 3321–3338.
- Tiwari S., Lata C., Chauhan P.S., Nautiyal C.S., 2016, *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery, *Plant Physiology and Biochemistry*, 99, 108-117.
- Yao L.X., Wu Z.S., Zheng Y.Y., Kaleem I., Li C., 2010, Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton, *European Journal of Soil Biology*, 46(1), 49-54.
- Zhang G., Sun Y., Sheng H., Li H., Liu X., 2018, Effects of the inoculations using bacteria producing ACC deaminase on ethylene metabolism and growth of wheat grown under different soil water contents, *Plant Physiology and Biochemistry*, 125, 178-184.
- Zhou C., Ma Z., Zhu L., Xiao X., Xie Y., Zhu J., Wang J., 2016, Rhizobacterial strain *Bacillus megaterium* BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance, *International Journal of Molecular Sciences*, 17(6), 976.