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# Can plant bio-regulators minimize crop productivity losses caused by drought, salinity and heat stress? An integrated review

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# Summary

Plant bio-regulators (PBRs), are biochemical compounds stimulates plant growth and productivity when applied, even in small quantities at appropriate plant growth stages. These are being extensively used in agriculture to enhance the productivity particularly in horticultural crops but are not as prevalent in field crops. Their central role in plant growth and development is through nutrient allocation and sourcesink transitions while most of the PBRs stimulate redox signaling under abiotic stress conditions. Since climate change and degrading natural resources are projected to amplify the stresses, particularly soil moisture deficit, high temperature and soil salinity, PBRs are likely to play a crucial role in plant growth regulation. However, the utility of PBRs to enhance crop productivity under stresses induced by abiotic factors needs critical evaluation. Research efforts so far have centered on the crop and agro-ecosystem specificity, optimal doses and schedule of their application for optimizing crop yields under stress conditions. These efforts are being complemented by investigations on genes and gene regulatory network at molecular level to tailor crop plants for climate resilience. In addition to complying with regulation governing use of bio-chemicals, issues related to crop yield losses in case of excessive doses as well as their impacts on soil health are being addressed. In this review, prospects and pathways of PBRs are thrashed out as an emerging stress alleviating technology for crop production in harsh agro-ecosystems, specifically those featured by drought, heat and salinity stress.

### Introduction

Understanding the adaptive responses of plants is of paramount importance particularly when grown under harsh environments. A series of experiments conducted in the past have clearly established that plant growth regulators and hormones play a vital role in determining growth, development and productivity of crops. This fundamental knowledge served as basis for a number of biochemical as plant growth promoters in crop production. This tends to be the integral part of modern agriculture, which is often affected by adverse environmental factors such as drought, high temperature and salt stress. In addition, recent surge in low external input sustainable agriculture (LEISA) demands enhanced intervention of modern plant biology that can integrate knowledge of plant responses to environmental factors at molecular, cellular, whole plant as well as cropping system levels.

Through LEISA, the plant stress tolerance can be improved with an exogenous use of stress alleviating chemicals (WAHID and SHABBIR, 2005; WAHID et al., 2007b; FAROOQ et al., 2009). Plant bioregulators (PBRs) are powerful tools for maximizing yield and quality, and increasing net income to farmers. PBRs have been used from

many years to alter the behavior of agricultural and horticultural crops for the economic benefit of the grower. Control of vegetative vigor, stimulation of flowering, regulation of crop load, reduction of fruit drop, and delay or stimulation of fruit maturity and ripening are best examples of regulation with the exogenous applications. Novel PBRs with possible benefits for fruit growers are continually being made available by industries. In addition, research is in progress to find new uses for bioregulator products that have been made available for specific uses.

Every aspect of plant growth and development is controlled by plant hormones and these serve as key integrators of exogenous (environmental) and endogenous (developmental) cues. The classes of phytohormones are auxins, gibberellins (GA), cytokinins, ethylene, abscisic acid (ABA), brassinosteroids (BS), salicylic acid (SA), and jasmonates, with strigolactones representing a relatively new addition (STAMM et al., 2011). Stress may induce common responses such as enhancement of plant hormones. For instance, wounding can induce increased production of ethylene, auxin, and ABA. Since many kinds of stresses including water, salt, and temperatures, induce ABA synthesis since ABA is considered as a plant stress hormone. It regulates several important aspects of plant growth and development. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptive responses for plants in adverse environmental conditions. ABA is also involved in several other physiological processes such as stomatal closure, embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids, germination, leaf senescence, and defense against pathogens. Nevertheless, ABA acts as a mediator in controlling adaptive plant responses to environmental stresses. In several instances, it has been implicated in signal transduction at the single-cell level. Other than plant hormones, exogenous PBRs such as inorganic, organic chemicals and booster's application will also have strong impact on plant adaptation to abiotic stress either independently or synergistically with one another (SRIVASTAVA et al., 2016). Many PBRs influence with the signaling pathways of one another thereby promoting plant tolerance to abiotic stress. Various researchers established the interlinking of redox signaling pathway among different PBRs to accomplish the goal of tolerance (SRIVASTAVA et al., 2016). The coordination of the responses triggered by the multiple stimuli is controlled by a network of intricate signal transduction pathways. This network has many signaling components, and a small number of highly interconnected components which are central for the functioning of the network (HETHERINGTON and WOODWARD, 2003). For instance, in many studies nitric oxide (NO) has been found to mediate the action of stomatal closure of ABA (GARCIA-MATA and LAMATTINA, 2007). PBRs also promote the uptake and metabolism of nutrient elements as well. Among the stress alleviating compounds, thiourea is one of the important molecule with two functional groups; 'thiol' is important to oxidative stress response and 'imino' partly fulfills the nitrogen requirement. Apart from thiol compounds other chemicals including plant growth regulators – 6-benzyladenine (BA), prohexadione-calcium (Pro-Ca), N-2-chloro-4-pyridinyl-N-phenylurea (CPPU), 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2pyridyloxyacetic acid (3,5,6-TPA), and amino ethoxy vinyl glycine (AVG), Dithiothreitol (DTT), Potassium Nitrate (KNO<sub>3</sub>), Thiourea (TU), Salicylic Acid (SA), Silicon (Si) products were used, to a small extent, to screen for their ability to increase agricultural yield without compromising quality.

Stressful environments interrupt the balance between generation and utilization of reactive oxygen species (ROS) leading to toxicity by enhancing production of ROS such as superoxide radicals ( $O_2^{-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radicals (OH<sup>-</sup>) etc. in plants thereby creating a state of oxidative stress in them (PANDA et al., 2003a, b). This increased ROS level in plants cause oxidative damage to bio-molecules such as lipids, proteins and nucleic acids, thus altering the redox homeostasis (SMIRNOFF, 1993; GILLE and SINGLER, 1995; SRIVASTAVA et al., 2016). When applied exogenously at suitable concentrations PBRs enhance the efficiency of antioxidant system, upregulates osmolytes and enhance the expression of stress responsive gens in plants (SRIVASTAVA et al., 2016; KNORZER et al., 1999) (Fig. 1) and we also proposed PBRs for sustainable agriculture by integrating redox signaling as a possible unifying mechanism (SRIVASTAVA et al., 2016).

In the current scenario, various kinds of PBRs such as TU, SA, Si, NO, H<sub>2</sub>O<sub>2</sub>, hydrogen rich water, BS and polyamines (PA) etc., have been tested for enhancing the plant stress tolerance as well as the crop yield (JISHA et al., 2013; SRIVASTAVA et al., 2016). Most of these PBRs treatments are applied either for seed priming or as foliar spray. Seed priming is a pre-sowing treatment that partially hydrates seeds without allowing the radicle emergence. The seed priming may be induced alone or in combination with the foliar application of PBRs at the time or early flowering or grain filling stage. The mechanism of seed priming mediated action is not well understood, but it may act in two ways. First, seed priming sets in motion germination-related activities (e.g. respiration, endosperm weakening, and gene transcription and translation, etc.) that facilitate the transition of quiescent dry seeds into germinating state and lead to improved germination potential. Secondly, priming imposes abiotic stress on seeds that represses radicle protrusion but stimulates stress responses e.g. accumulation of late embryogenesis abundant (LEAs), potentially inducing cross-tolerance (CHEN and ARORA, 2013). These two strategies together also impose a 'memory'

in seeds, which can be recruited upon a subsequent stress-exposure and may mediate a greater stress-tolerance in the subsequent generation (PASTOR et al., 2013).

The specific influence of exogenous PBRs on crop plants has already been integrated into the crop production systems; e.g. the use of chlormequat chloride (CC) in cereals crop growth, ethephon (ETH) for influencing the development and maturity of various crops and mepiquat chloride in cotton. Several such PBRs also may be useful in overcoming the production constraints. Foliar application of synthetic auxin - naphthal acetic acid (NAA) to enhance apple flower (CLINE, 2006a; HARLEY et al., 1958; MCARTNEY et al., 2007; STOVER et al., 2001) and also documented as flower-promoter in several tree fruit crops (COWGILL and AUTIO, 2009; SCHWALLIER, 2006; WASHINGTON STATE UNIVERSITY EXTENSION, 2009). ETH has also enhanced flowering in bearing apple trees (CLINE, 2006a). The PBR prohexadione-calcium (P-Ca), an inhibitor of gibberellic acid (GA) biosynthesis reduces shoot extension in several fruit tree species (RADEMACHER et al., 2006). Exogenous cytokinin application of benzyladenine (BA) has effectively improved lateral branching of nursery trees (HROTKO et al., 1997) by limit blindwood and improves canopy development.

### Plant bio-regulators (PBRs)

The most commonly used PBRs, include various plant hormones (FAROOQ et al., 2015; RATNAKUMAR et al., 2015a, b; SRIVASTAVA et al., 2016) thus, affect plant's ability to respond to its environment (Tab. 1 and 2). They interact with specific target tissues to cause physiological responses, such as growth and development. Each response is often the result of two or more hormones acting together. Among them, many hormones can be synthesized in the laboratory, therefore increasing the quantity of hormones available for commercial applications. For instance, GA is widely used as PBRs. Likewise, SA is another important plant hormone that regulates a wide range of metabolic and physiological responses in plants such as seed germination, seedling establishment, cell growth, respiration, stomatal closure, senescence-associated gene expression, responses to abiotic stresses, basal thermo-tolerance, nodulation in legumes, and fruit yield. Hence they are used as PBRs for enhancing plant growth and yield (VLOT et al., 2009; VICENTE and PLASENCIA, 2011) (Tab. 2) and for particular stress alleviation (Tab. 1). However, judicious use of SA dose is required as the excess concentration proves

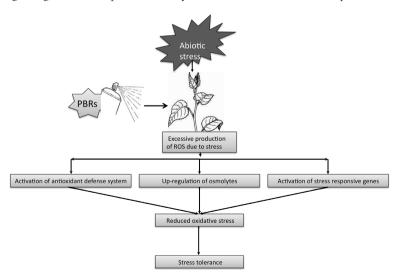


Fig. 1: Schematic representation of abiotic stress tolerance mechanism induced by plant bio-regulators (PBRs) in holistic level; impact on antioxidative system, up-regulation of osmolytes and gene activation. Owing to their ability to modulate ROS level, upregulation of osmolytes and activation of stress responsive genes, PBRs help to establish redox homeostasis which either avaoids or minimizes the plant ability to combat stress induced redox imbalance.

Stress	Plant	PBRs	Studied parameters	Response	Reference
Drought	Wheat     SA     Content of ascorbate and glutathione		+	KANG et al. (2013)	
	Wheat	SA	Moisture content, dry matter accumulation, carboxylase activity of Rubisco, SOD and total chlorophyll	+	SINGH and USHA (2003)
	Barley	SA	ABA content in leaves	+	BANDURSKA and STROINSKI (2005)
	Wheat	SA	Stomatal regulation, maintaining leaf chloro- phyll content, increasing water use efficiency, and stimulating root growth	+	ANOSHEH et al. (2012)
	Salvia officinalis	SA	Remobilization of stored food	+	ABREU and MUNNE-BOSCH (2008)
	Phaseolus vulgaris	PGPR	Production of IAA, cytokinins, antioxidants and ACC deaminase	+	FIGUEIREDO et al. (2008)
	Wheat	SA	Chlorophyll pigments and chlorophyll a/b ratio	-	MOHAREKAR et al. (2003)
	Wheat	Thiourea	Individual grain weight	+	SAHU and SINGH (1995)
Heat	Arabidopsis	SA	Oxidative stress	+	ALONSO-RAMÍREZ et al. (2009)
	Mustard	SA	H <sub>2</sub> O <sub>2</sub> content and CAT activity	+ and -	DAT et al. (1998)
	Agrostis stolonifera	SA	POX activity and CAT activity	+ and -	LARKINDALE and HUANG (2004)
	Cicer arietinum	SA	Protein and proline contents induction of various stress enzymes <i>viz</i> . POX and APX	+	CHAKRABORTY and TONGDEN (2005)
	Wheat	SA	Proline content	+	KHAN et al. (2013)
Salt	Wheat	SA	IAA and cytokinin levels, Proline content, ABA accumulation, SOD and POX activity	+	SHAKIROVA et al. (2003)
	Wheat	SA	osmotic potential, shoot and root dry mass, K <sup>+</sup> /Na <sup>+</sup> ratio and photosynthetic pigments content	+	KAYDAN et al. (2007)
	Barley	SA	Photosynthetic rate, membrane stability	+	El Tayeb (2005)
	B. juncea	SA	Growth, photosynthetic parameters and activities of enzymes (nitrate reductase, carbonic anhydrase, CAT, POX and SOD), Proline content	+	YUSUF et al. (2008)
	Mungbean	SA	Antioxidant system	+	KHAN et al. (2014)
	Tomato	SA	Activation of aldose reductase and ascorbate peroxidase, accumulation of osmolytes such as proline	+	TARI et al. (2002, 2004), SZEPESI et al. (2005)

Tab. 1: Drought, heat stress and salinity induced plant tolerance by plant bio-regulator (PBRs) viz., SA (salicylic acid), TU (thiourea) and PGPR (plant growth promoting rhyzobactria). The indication marks '+' represents the positive and '-' represents negative responses.

toxic for the plant growth (DONOVAN et al., 2013). Exogenous application of cytokinin, preferably kinetin, to the foliage of plants has also been shown to increase crop productivity. The application of low concentrations of potassium together with cytokinin provides synergistic effect (US PATENT, 2012). Similarly, early application of trehalose has also shown to enhance the health and vigor of plant resulting in better production of sugar content (US PATENT, 2013). The application of plant sterols and steroid hormones such as brassinosteroids (BS) are essential for plant growth, reproduction and responses to various abiotic and biotic stresses. The use of these BS (VRIET et al., 2012) has also been proposed as a promising strategy for crop improvement.

#### **Biotic agents**

Apart from chemical based PBRs, various biotic agents (Tab. 2) have also been used for the same purpose. NAGARAJU et al. (2012) have used *Trichoderma harzianum* isolates to enhance the plant growth and resistance of sunflower towards downy mildew disease. Many bacteria have been found to not only promote the growth of plants but also protect the plants against various abiotic and biotic stress agents including flooding, drought, salts, metals, organic contaminants, wilting and pathogens (GLICK, 2014).

### Effects of PBRs on crop growth

Salicylic acid (SA) and other salicylates, thio-urea and other thiol compounds, KNO<sub>3</sub> and other nitrites, are known to affect various physiological and biochemical activities of plants and may play a key role in regulating plant growth and productivity (ARBERG, 1981). Studies have provided conflicting evidences regarding role of SA in flowering. However, some demonstrations argue the role of SA in flowering. In SA-deficient *Arabidopsis* initiation of flowering failed when irradiated with UV-C and substantial flowering occurred when grown under non-stress condition than wild-type plants (SHULAEV et al., 1995). SIZ1, a SUMO E3 ligase, negatively regulates flowering via an SA-dependent pathway (JIN et al., 2008). It was reported that the dry matter accumulation was significantly enhanced in *Brassica juncea*, with SA foliar application. However, higher concentrations of SA had an inhibitory effect. In sugarcane GA3 increased

Tab. 2:	Chemical-based PBRs	and biotic agents, their	mode of action in cro	p plants and orchards.
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PBRs	Type of Plant	Mode of action	References	
Chemical based PBRs				
TU, SA, Si, NO, H <sub>2</sub> O <sub>2</sub> , hydrogen rich water, Brassinosteroids and Polyamines	Crop plants	Improves plant stress tolerance and crop yield	SRIVASTAVA et al., 2016	
Chlormequat chloride (CC); Ethephon (ETH) and Mepiquat chloride	Cereals, cotton	Crop growth, development and maturity of various crops	JISHA et al., 2013; SRIVASTAVA et al., 2016	
Naphthal Acetic Acid (NAA) and ETH	Apple and several tree fruit crop	Enhance flower and known as flower promoter	HARLEY et al., 1958; MCARTNEY et al., 2007; STOVER et al., 2001; CLINE, 2006a	
Prohexadione-calcium (P-Ca),	Several fruit tree species	Inhibitor of gibberellic acid (GA) biosynthesis reduces shoot extension	RADEMACHER et al., 2006	
Benzyladenine (BA)	Nursery trees	Improved lateral branching	Нкотко et al., 1997	
Trehalose		Enhance the health and vigor of plant production of sugar content	US PATENT, 2013	
Brassinosteroids (BS)		plant growth, reproduction and responses	VRIET et al., 2012	
Potassium (K)	Plant cell	osmosis regulation and photosynthesis	Arteca, 1995	
Aminocyclopropane-1-carboxylate (ACC, the precursor of ethylene)		transcription of genes that encode plant defensive/protective proteins	ROBISON et al., 2001	
Thio-urea (TU)	Wheat	Grain yield	SAHU et al., 2006	
Brassinosteroids (BS)		Hypocotyl cell elongation involves a microtubule regulatory protein, Microtubule destabilizing protein 40	WANG et al., 2012b	
Thio-urea (TU)	Wheat	Improved productivity and enhanced grain weight	SAHU and SINGH, 1995	
SA and SNP (Nitric oxide source)	-	Promoted Fe uptake, translocation and activation; modulated the balance of mineral elements; and protect Fe deficiency	Kong et al., 2014	
Biotic Agents				
Pseudomonas asplenii Phragmites australis seeds		Improved germination and protected the plants	BASHAN et al., 2008	
Purple phototrophic bacteria (PPB) Stevia rebaudiana		Increased the growth and also yield of stevioside (ST)	WU et al., 2013	
PGPRs (plant growth promoting <i>rhizobacteria</i> )		Induce the production of auxin or inhibit ethylene synthesis or mineralization of nutrients	STEENHOUDT and VANDERLEYDEN, 2000	
Trichoderma harzianum Sunflower		Plant growth and resistance downy mildew	NAGARAJU et al., 2012	
Pseudomonas fluorescens		Promote plant growth	GLICK, 2014	

inter-nodal elongation, while glyphosate, CEPA and other regulators increase the deposition of sucrose, diquat and 2-chloroethylphosphonic acid (CEPA) inhibit flowering, and paraquat desiccates leaves just prior to harvest to facilitate leaf removal or burning (KOSSUTH, 1984).

GA increased the rate of cell division and stimulation of vegetative growth (ARTECA, 1995). Leaf area was found to increase significantly after KNO<sub>3</sub> application probably due to promoting role of potassium (K) in plant growth. Generally the essential element K has a great regulatory role within plant cells and organs such as, activating on enzymes, osmosis regulation and photosynthesis and loading and unloading of sugars in phloem (osmosis regulation and photosynthesis). DCPTA delay the natural senescence of mature leaves thereby contributing to enlarged leaf canopies and improved carbon assimilation per unit leaf area. Flowering is another important parameter is directly related to yield and productivity of plants. It is well known that SA application induces flowering in a number of plants (CLELAND and AJAMI, 1974). However, the rigorous mechanism of flower inducing property of SA is not been explored. KUMAR et al. (2000) studied the cumulative effect of SA in combination with GA, Kinetin, NAA, ethral and chloro chloro chloride (CCC), and found a synergistic effect between SA and GA on flowering as compared to other combinations of hormones.

Likewise, ethylene modulates plant growth and development under normal conditions and is also a key feature in the response of plants to a wide range of stresses. Ethylene is synthesized in plants in response to various stresses typically in response to the presence of metals, organic and inorganic chemicals, cold or heat stress, drought or flood, ultraviolet light, insect and nematode damage, phyto-pathogens (both fungi and bacteria), and mechanical wounding (GLICK, 2014). A small fraction ethylene synthesis, which consumes the existing pool of 1-aminocyclopropane-1-carboxylate (ACC, the precursor of ethylene) in stressed plant tissues, is believed to be responsible for initiating the transcription of genes that encode plant defensive/ protective proteins (ROBISON et al., 2001). However, higher level of ethylene production following synthesis by the plant of additional ACC in response to a stress is generally injurious to plant growth and usually involved in initiating senescence, chlorosis and leaf abscission (GLICK, 2014).

There has been a major increase in the utilization of BRs in agricultural applications as a mean to boost crop productivity and stress tolerance. Crosstalk and interactions between BRs and other plant growth regulators occur through either the modification or intersection of their primary signaling cascades and function to regulate a large and diverse array of biological processes (CHOUDHARY et al., 2012). ABA regulates both stress- and non-stress-related plant responses by acting as a signal molecule. Interactions between BRs and ABA regulate the expression of many genes that govern several biological processes, such as seed germination, stomatal closure and plant responses to environmental stresses.

Interaction between soil bacterium may be negative, positive or neutral and sometimes the effect of soil bacterium changes according to the conditions of soil. For instance, when the availability of chemical nitrogen fertilizer is abundant in soil, a nitrogen fixing bacterium is useless for the plants. Similarly many bacteria are useful for the plants only under environmental stress conditions and are unlikely to have any beneficial effect under optimal conditions (GLICK et al., 2007). It is important to mention here that, not all strains of a particular bacterial genus or species promote plant growth due to varied genetic makeup and metabolic capabilities. Some strains of Pseudomonas fluorescens, for instance may actively promote plant growth while other strains of this species have no measurable effect on plants (GLICK, 2014). Plant growth-promoting bacteria may facilitate plant growth and development either directly or indirectly. The indirect effect on promotion of plant growth occurs by preventing harmful effects of plant pathogens and direct role of the PGBRs involves either acquisition of mineral nutrients or modulation of plant growth by alteration of phytohormones such as auxin, cytokinin and ethylene (GLICK, 2012). Bacteria control the level of ethylene production in some plants through the auxins they produce; the most commonly observed mechanism employed by bacteria that diminish levels of ethylene production is via the activity of bacterial 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase). Bacteria of different origins expressing ACC deaminase activity (leading to a decrease in ACC levels and thus in ethylene production) can stimulate plant growth even in soils containing phytotoxic concentrations of cadmium. It has been found that most of the plant growth-promoting rhizobacteria isolated from Graminaceae grasses growing in a meadow polluted with heavy metals exhibited ACC deaminase activity, which resulted in plant growth promotion (DELL'AMICO et al., 2005). Some of the abiotic stresses whose effects can be ameliorated in this way include temperature extremes, flooding, drought, metals and metaloids, hypoxia, salt and organic contaminants (GLICK, 2012). Foliar spray and rhizosphere irrigation with purple phototrophic bacteria (PPB) on a herb Stevia rebaudiana native to certain regions of South America increased the growth and also yield of Stevioside (ST), one of the steviol glycosides, which is 300 times sweeter than cane sugar and non-calorific, and has specific immunomodulatory activities (WU et al. 2013).

### Germination

SA is known to improve germination, plant growth, rate of transpiration, stomatal regulation, photosynthesis, ion uptake and transport in plants (METWALLY et al., 2003; KHODARY, 2004; HE et al.,

2010; KHAN et al., 2014, 2015). Low doses of SA have potential to improve seed germination and seedling establishment under different abiotic stress conditions (RAJJOU et al., 2006; ALONSO-RAMÍREZ et al., 2009). LEE et al. (2010) reported the role of SA in seed germination under salinity. They found that SA promotes germination under high salinity by modulating antioxidant activity in Arabidopsis. Further, they suggested that, SA was not essential for germination under normal growth conditions, but under saline conditions it promotes seed germination by reducing oxidative damage. Similarly, HANIEH et al. (2013) determined the effect of pre-sowing treatment of SA on seed germination of sweet pepper and found that, pre-sowing seed treatment of SA caused better germination percentage and faster growth rate. Proteomic analyses showed that two SODs are induced by SA in Arabidopsis germinating seeds, which might contribute to an enhanced antioxidant capacity (RAJJOU et al., 2006). For instance, about 0.5 mM SA treatment for 24 h caused a strong up-regulation of translation initiation and elongation factors, proteases, and two subunits of the 20S proteasome. This supports the hypothesis that SA improves seed germination by promoting the synthesis of proteins that are essential for germination, and the mobilization or degradation of seed proteins accumulated during seed maturation (RAJJOU et al., 2006). BR-related mutants (det2-1 and bri1-1) showed increased sensitivity to the inhibitory effects of ABA during seed germination in comparison with wild type plants (STEBER and MC-COURT, 2001). However, primary root and hypocotyl elongation assays in the sax1 mutant revealed hypersensitive responses to ABA, as well as auxin and ethylene (EPHRITIKHINE et al., 1999). In Phragmites australis inoculation of seeds with Pseudomonas asplenii improved germination and protected the plants from growth inhibition (BASHAN et al., 2008).

### **Root growth**

Prolific root system is important for stress tolerance and improved water uptake particularly under abiotic stress conditions in order to harvest better crop yield (FAROOQ et al., 2009; FLOWERS, 2004) under abiotic stress conditions. Normally sensitive genotypes have poor prolific roots than susceptible genotypes. Corresponding to this, a pronounced increment in root growth of sensitive wheat varieties to salt and high temperature stress was reported with the application of TU and that strongly correlate to grain yield (SAHU et al., 2006). Thus, in view of TU being water soluble, readily absorbable in the tissues that enhance the plant, it has ability to ameliorate stress effects under field conditions.

Superiority of synthetic growth hormone NAA over other bioregulators attributed its unique role in delaying senescence process, hastening root and shoot growth and setting more fruits (WEIN et al., 1989). KEITHLY et al. (1990) reported that photosynthate partitioning in DCPTA-treated (foliar application) sugar beet plants appeared to be balanced between the demands of plant growth and taproot sucrose accumulation supply. Increased taproot weight of 30  $\mu$ M DCPTAtreated taproots resulted in an 81% increase in sucrose yield.

SA increased the level of cell division within the apical meristem of seedling roots of wheat plants (SAKHABUTDINOVA et al., 2003). Similarly, applications of  $10^{-8}$  and  $10^{-6}$  M SA increased root length (SAN-MIGUEL et al., 2003). The exogenous application of SA to plants results in an interference with the ion transportation and absorption in the membranes of root cells (HARPER and BALKE, 1981). In soybean plants treated with 10 nM, 100  $\mu$ M, and up to 10 mM SA, root growth increased up to 45% (SHAKIROVA et al., 2003).

The influence of BSs on PIN Aux efflux carriers, which control mitotic activity and cell differentiation, suggests a possible mechanism that contributes to BS-mediated root growth through regulation of Aux distribution (CHOUDHARY et al., 2012). BSs were unable to antagonize the ET effects on hypocotyl growth in the etiolated *fer-2*  mutant, indicating FER-dependent BS effects on ET-induced growth responses (DESLAURIERS and LARSEN, 2010; CHEUNG and WU, 2011). BR-mediated hypocotyl cell elongation involves a micro-tubule regulatory protein, Microtubule destabilizing protein 40 (MDP40) (WANG et al., 2012b).

In some plants, the treatment of seeds or cuttings with non-pathogenic bacteria, such as *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Streptomyces*, etc., induces root formation (ESITKEN et al., 2003). PGPRs (plant growth promoting *rhizobacteria*) might induce the production of auxin or inhibit ethylene synthesis or mineralization of nutrients (STEENHOUDT and VANDERLEYDEN, 2000). DESBROSSES et al. (2009) investigated the PGPR-*Arabidopsis* interaction to establish the signaling pathways involved in controlling plant development and observed an ethylene-independent and auxin-independent mechanism, regulating the elongation of root hair in Arabiopsis.

# Nutrient uptake, mobilization and translocation

Application of NAA (20 ppm) produced mean maximum yield of 17.60 and 47.05 q ha<sup>-1</sup> of seed and straw of fenugreek, respectively and was found to be superior to other bio-regulators like GA. N, P and K content. Their uptake in seed and straw were also the maximum with 20 ppm NAA treatment which was N: 5.50, P: 8.14, K: 2.20 and N: 5.81, P: 12.86, K; 1.94 percent high over water sprayed in seed and straw of fenugreek, respectively. It was also documented that hormone application increases physiological and metabolic activities of the plant as a result more uptake of nutrients by plants from the soil (NICKELL, 1982). It was also perceived that higher concentration of BS (i.e. 0.5 ppm) increases yield, NPK content and uptake over GA, due to homo-brassinosteroid synergistic interaction with the endogenous auxin. (CRANE, 1944). The favorable effect of plant growth regulators in enhancing the yields and nutrient uptake was also reported in cotton (PATEL, 1992) and green gram (TAKAHASHI, 1994), respectively. Nitric oxide (NO) or its donor has been shown to mediate chlorophyll increase, Fe availability and antioxidant enzymes (ZHANG et al., 2012). Several models suggest that redox signalling through NO and ROS is enhanced by SA in a self-amplifying process. However, the relationship between NO, SA, and ROS in the activation of defense genes and/or induction of host cell death is not clearly defined (ZOTTINI et al., 2007). SA and SNP (Nitric oxide source) promoted Fe uptake, translocation and activation; modulated the balance of mineral elements; and protected Fe deficiency induced oxidative stress (KONG et al., 2014) (Tab. 2). Little information is available on the role of BRs under nutrient deficient conditions. WANG et al. (2012) reported that BRs play a negative role in regulating Iron (Fe)-deficiency-induced ferric reductase (FRO), expressions of CsFRO1 (transcripts encoding FRO) and CsIRT1 (Fe transporter), as well as Fe translocation from roots to shoots.

In wheat, the mobilization flow rate of sucrose to grain determines the rate at which carbohydrate can accumulate in the ear (JENNER and RATHJEN, 1972). SAHU and SINGH (1995) reported that improved productivity of wheat under soil and foliar treatments of thio-urea (TU) was mainly due to enhanced grain weight. Metabolic transport of sucrose to grains via effects on phloem loading was enhanced with application of TU. Mobilization of dry matter (reserves) from leaves to grains increased in wheat with TU spray at tiller stage. Improvement in harvest index under TU treatments lends further credence to the role of TU in improving dry matter partitioning to grains (SAHU and SOLANKI, 1991). Reduced grain yield with 20 kg/ha soil-applied TU compared with 10 kg/ha treatment might be attributed to inhibitory effects perhaps on phloem transport of sucrose (UPPAL, 1986) The role of TU as a thiol compound and growth-regulating chemical for improving the productivity of field crops such as wheat and brassica were studied extensively (SRIVASTAVA, 2010; SAHU, 2006; NIASM, 2013) (Tab. 1). Inoculation of rhizobacteria increased uptake of nutrient elements like Ca, K, Fe, Cu, Mn and Zn by plants through stimulation of proton pump ATPase (MANTELIN and TOURAINE, 2004; KUMAR et al., 2014). In crop plants, mineral elements uptake also increased in combined inoculants of *Bacillus* and *Microbacterium* (KARLIDAG et al., 2007). PGPRs might increase the nutrient uptake of plants through organic acid production and decreasing the soil pH in rhizosphere.

# PBRs induced plant adaptation and mitigation under drought, salinity and heat stress

### Drought

Exposure of plants to water stress leads to serious physiological and biochemical dysfunctions including reduction in turgor, growth, photosynthetic rate, stomatal conductance and damages of cellular components (JANDA et al., 2007). SA has significant role in controlling abiotic stresses including drought and salinity stress. SA has high potential for improving stress tolerance in agriculturally important crops. Its utility, however depends on various factors like concentration of SA applied, mode of application and the stage of plant growth. At low concentrations SA has been found to alleviate abiotic stress and at higher concentrations it induces oxidative stress (VICENTE and PLASENCIA, 2011; MIURA and TADA, 2014). Higher tolerance to drought stress was also observed in the plants raised from the grains soaked in aqueous solution of acetyl salicylic acid (HAMADA, 1998; HAMADA and AL-HAKIMI, 2001). It has been suggested that SA-induced drought tolerance is associated with an enhanced antioxidant system (HORVÁTH et al., 2007; MUTLU et al., 2009; ZHOU et al., 2009). However, very little is known about the molecular mechanisms of SA-induced drought or other abiotic tolerances in higher plants. A few studies have shown that abiotic tolerance induced by SA could be related to the altered expression of the genes encoding osmotin, pathogenesis-related proteins, and heat shock proteins (DING et al., 2002; KIM and DELANEY, 2002; CLARKE et al., 2004). To explore the molecular mechanisms involved an effort was made by KANG et al. (2013). They found that treatment with 0.5 mM salicylic acid (SA) significantly alleviated growth inhibition induced by drought in wheat seedlings by significantly increasing the content of ascorbate (ASA) and glutathione (GSH) due to enhanced transcription of GST1, GST2, glutathione reductase (GR), and mono-dehydro ascorbate reductase (MDHAR) genes (KANG et al., 2013). Treatment with SA increased drought tolerance of common bean (Phaseolus vulgaris) and tomato (Solanum lycopersicum) plants (SENARATNA et al., 2000). Exogenously applied SA has also been reported to modulate activities of intracellular antioxidant enzymes SOD and POD and increase plant tolerance to environmental stresses (SAKHABUTDINOVA et al., 2004; SENARATNA et al., 2000). Application of SA alleviated adverse effects of drought stress in wheat plants by increasing improving stomatal regulation, maintaining leaf chlorophyll content, increasing water use efficiency, and stimulating root growth (ANOSHEH et al., 2012). Leaf senescence is a highly regulated physiological process, allowing the remobilization of stored food from the older leaves to the rest of the plant, during stressful conditions and SA involved in the promotion of drought-induced leaf senescence in Salvia officinalis plants (ABREU and MUNNE-BOSCH, 2008). The results reported by SINGH and USHA (2003) revealed that the wheat seedlings subjected to drought stress when treated with SA generally exhibited higher moisture content and also higher dry matter accumulation, carboxylase activity of Rubisco, SOD and total chlorophyll content compared to the untreated plants. Exogenous application of SA also alleviated the damaging effects of water deficit on cell membranes of barley plants and concomitantly increased ABA content in leaves, which might have contributed to the enhanced tolerance of plants to water scarcity (BANDURSKA and STROINSKI, 2005). Besides providing tolerance to

plants against drought stress, the exogenous application of SA was also found to be effective in providing resistance to the plants against the excessive water stress as was observed in cell suspensions prepared from the fully turgid leaves of *Sporobcdus stapfianus* (GHASEM-POUR et al., 2001). The impact of thiourea in improving productivity of wheat (SAHU and SINGH, 1995), showed individual grain weight significantly improved under soil and foliar treatments of TU.

It is a well-established fact that SA potentially generates a wide array of metabolic responses in plants and also affects the photosynthetic parameters and plant water relations. However, contrary to these observations, a reduction in chlorophyll content was observed in plants pre-treated with SA (ANANDHI and RAMANUJAM, 1997; PANCHEVA et al., 1996). MOHAREKAR et al. (2003) reported that SA activated the synthesis of carotenoids and xanthophylls and also enhanced the rate of deep oxidation with a concomitant decrease in chlorophyll pigments and chlorophyll a/b ratio in wheat. However in contrast, the transpiration rate decreased significantly in Phaseolus vulgaris and Commelina communis after the foliar application of SA. That decrease in transpiration rate was attributed to the fact that SA induced the closure of stomata (LARQUE-SAAVEDRA, 1978, 1979). SA was also shown enhance photosynthesis and growth of soybean (C<sub>3</sub> plant) and corn (C<sub>4</sub> plant) under greenhouse conditions (KHAN et al., 2003). SA also has capacity of osmotic adjustment by maintaining low MDA contents and decreased Na+/K+ ratio in leaves (FAYEZ and BAZAID, 2014). Brassinosteroids enhance tolerance to drought and cold stress by modulations of the expression of drought- and cold-stress marker genes (KAGALE et al., 2007). However, the exact biochemical link between the Brassinosteroids -signal cascade and stress tolerance remains a mystery.

Both soil and foliar treatments of thio-urea (TU) increased the number of ears and grains/ear, indicating an improved storage capacity. In this context, it is noteworthy that di-thiothreitol, a thiol containing two -SH groups, stimulated carbon dioxide assimilation in the dark up to five-folds (WERDAN et al., 1975). Because of its cytokine-like activity, TU might have also delayed leaf senescence (HALMANN, 1990). In maize, foliar spray of TU increased both canopy photosynthesis and photosynthetically active leaf surface during grain filling (SAHU et al., 1993). Chloroplasts isolated from mature leaves of 30 µM DCPTA-treated plants, as compared with that of controls, showed a 23% increase in the total soluble protein to chlorophyll ratio. This parallels to an observed increase in activated ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activity in vitro per unit chlorophyll. The Rubisco activity increased 87% per dm<sup>2</sup> leaf area of 30 µM DCPTA-treated plants. Increased Rubisco activity largely accounted for increase in net photosynthesis in DCPTAtreated plants. However, intense sink demand for photosynthate and the delayed leaf senescence of older leaves may increase net-carbon assimilation in mature (source) leaves of DCPTA-treated plants.

Tolerance to drought stress is enhanced in PGPR inoculated plants (FIGUEIREDO et al., 2008). Plants achieve this tolerance either due to the production of IAA, cytokinins, antioxidants and ACC deaminase. Reports are also available regarding role of PGPR in conferring resistance to water stress in plants such as tomatoes and peppers under water deficient conditions (AROCA and RUIZ-LOZANO, 2009). More efforts are needed to investigate the mechanistic approach of PGPR in eliciting tolerance to different stresses. This would improve our understanding of induced systemic tolerance to water stress in modern agriculture.

### Salinity

High salinity induces serious metabolic perturbations in plants, as it generates ROS which disturb the cellular redox system in favor of oxidized forms thereby creating an oxidative stress that may damage DNA, inactivate enzymes and cause lipid peroxidation (SMIRNOFF,

1993) (Tab. 1). The results of SRIVASTVA et al. (2009) recommend that TU treatment maintains the integrity and functioning of mitochondria in seeds as well as seedlings exposed to salinity. Thus, TU has the potential to be used as an effective bioregulator to impart salinity tolerance under field conditions, and might prove to be of high economic importance by opening new avenues for both basic and applied research. However, most of the literature indicates that exogenous application of SA to the stressed plants can potentially alleviate the toxic effects, generated by salinity. An enhanced tolerance against salinity stress was observed in wheat seedlings raised from the grains soaked in SA (HAMADA and AL-HAKIMI, 2001). Similar observations were also made in tomato plants raised from the seeds soaked in SA and was presumed to be due to the enhanced activation of some enzymes viz. aldose reductase and ascorbate peroxidase and to the accumulation of certain osmolytes such as proline (TARI et al., 2002, 2004; SZEPESI et al., 2005). Accumulation of large amounts of osmolytes (proline) is an adaptive response in plants exposed to stressful environments (RAI, 2002). Wheat seedlings accumulated large amounts of proline under salinity stress which was further increased with SA applied exogenously, thereby alleviating the deleterious effects of salinity (SHAKIROVA et al., 2003). The exogenous application of SA prevented the lowering of IAA and cytokinin levels in salinity stressed wheat plants resulting in the betterment of cell division in root apical meristem, thereby increasing growth and productivity of plants (SHAKIROVA et al., 2003). It is widely reported that the pre-treatment with SA resulted in the accumulation of ABA which might have contributed to the pre-adaptation of seedlings to salinity stress since; ABA induces the synthesis of a wide range of anti-stress proteins, thereby providing protection to the plants. Further, the treatment also lowered the level of active oxygen species and therefore the activities of SOD and POX were also lowered in the roots of young wheat seedlings (SHAKIROVA et al., 2003). These findings indicate that the activities of these antioxidant enzymes are directly or indirectly regulated by SA, thereby providing protection against salinity stress (SAKHABUTDINOVA et al., 2004). Exogenous application of SA enhanced the photosynthetic rate and also maintained the stability of membranes, thereby improved the growth of salinity stressed barley plants (EL TAYEB, 2005). The damaging effects of salinity were also alleviated by exogenous application of SA in Arabidopsis seedlings (BORSANI et al., 2001). KAYDAN et al. (2007) observed that pre-sowing soaking treatment of seeds with SA positively affected the osmotic potential, shoot and root dry mass, K<sup>+</sup>/Na<sup>+</sup> ratio and contents of photosynthetic pigments (chlorophyll a, b and carotenoids) in wheat seedlings, under both saline and nonsaline conditions. The loss of growth, photosynthetic parameters and the activities of enzymes (nitrate reductase and carbonic anhydrase) as a result of salinity stress in *B. juncea* was revived when SA was sprayed to the foliage, at 30 days stage. Further the activities of various antioxidant enzymes (CAT, POX and SOD) were increased with a concomitant increase in proline content (YUSUF et al., 2008). SA enhanced plant salt tolerance in terms of improving all measured growth parameters, photosynthetic efficiency and enhanced the antioxidant enzyme contents in response to NaCl and/or SA treatment providing a synergistic interaction (ISMAIL, 2013; KHAN et al., 2014). In both abscisic acid (ABA) levels increase in both drought and high salinity conditions, along with gene expression changes (ZELLER et al., 2009). Intensive crosstalk of ABA with different signaling pathways produces abundance of proteins and secondary messengers that act as regulators or modulators of ABA responses (CHRISTMANN et al., 2006). Both ABA and ethylene responses integrate at the level of DELLA proteins under salt stress and under conditions of high salinity and vegetative growth of plants occurs through activation of ABA and ethylene signaling. These two pathways eventually result in a DELLA-mediated inhibition of growth and a delay in flowering, respectively, which ultimately promotes plant survival (ACHARD

et al., 2006). This DELLA-mediated growth control and plant survival under high salt conditions was shown to be due to an increased accumulation of enzymes that detoxify reactive oxygen species (ROS) (ACHARD et al., 2008). BS crosstalk with SA regulates plant responses to abiotic stress. Exogenously applied BRs could not confer salt stress tolerance in the SA-insensitive *npr1-1* mutant in *Arabidopsis* (DIVI et al., 2010). This supports the idea that BR-induced salt tolerance in *Arabidopsis* partially depends on NPR1, a master regulator of the SA-mediated defense signaling pathway (DIVI et al., 2010). In *Brassica juncea* salt stress tolerance increased with exogenous applications of BS and SA. The combined application of BS and SA was most effective in alleviating the salt stress when compared with their individual treatments (HAYAT et al., 2012).

Other PGBRs which enhnce salt tolerance in plants include mycorrhizal associations of plants. Different mechanisms are employed by arbuscular mycorrhizal fungi to enhance salt tolerance of host plants like enhancing nutrient acquisition (P, N, Mg and Ca) (AZCON and ATRASH, 1997; GIRI and MUKERJI, 2004; SHENG et al., 2009), inhibition of high uptake of Na and Cl and their transport to plant shoots (DAEI et al., 2009), improving water uptake (RUIZ-LOZANO and AZCON, 2000), accumulating of proline and polyamines (EVELIN et al., 2009; IBRAHIM et al., 2011) and also by increasing some of enzymatic antioxidant defense system (SOD and CAT) (FAROOQ et al., 2015; WU et al., 2010) (Fig. 2). Arbuscular mycorrhizal association also improve osmotic adjustment, which helps in maintenance of the leaf turgor pressure, which in turn affects photosynthesis, transpiration, stomatal conductance and water use efficiency (JUNIPER and ABBOTT, 1993). Under saline condition, mycorrhizal inoculation of three different arbuscular mycorrhizal fungi, Glomus mosseae, G. deserticola and Gigaspora gergaria significantly increased growth responses, nutrient contents, acid and alkaline phosphatases, proline and total soluble protein of wheat plants compared to non-mycorrhizal ones (ABDEL-FATTAH and ASRAR, 2012). Foliar application of moringa (Moringa oleifera) leaf extract (MLE; 30 times diluted), benzyl amino purine (BAP; 50 mg L<sup>-1</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; 120 µM) at tillering, jointing, booting and heading growth stages decreased the shoot Na<sup>+</sup> and Cl<sup>-</sup> contents, with simultaneous increase in shoot K<sup>+</sup> contents (YASMEEN et al., 2013).

### Heat stress

Deviation from optimum temperature results in serious perturbations in plant growth and development. This may be due to membrane disruptions, metabolic alterations and generation of oxidative stress (MITTLER, 2002). Both salinity and high temperatures have a common facet of oxidative damage (WAHID et al., 2007a). Looking at the structure of thio-urea, both 'imino' and 'thiol' functional groups has great implications in abiotic stress tolerance. With foliar spray, amino group provides a ready source of nitrogen and thiol has a great role in alleviating oxidative stress damage on the physiologically more important mesophyll tissue. It was evident from the results that foliar applied thio-urea was effective in improving salt- (6-11%) and high temperature tolerance (4-10%) of wheat varieties. However, SA plays a key role in providing tolerance against temperature stress. SA stimulates alternative respiratory pathway in mitochondria by inducing expression of alternative oxidase, the terminal electron acceptor of the alternative respiratory pathway and releases unused potential energy as heat (VLOT et al., 2009). SA has been found to provide protection against heat stress in plants (KARLIDAG et al., 2009; KHAN et al., 2013). Exogenous application of SA also partially reverses the inhibitory effect of oxidative (0.5 mM paraquat) and heat stress (50 °C for 3 h) on seed germination (ALONSO-RAMÍREZ et al., 2009). Jasmonic acid also acts with SA, resulting basal thermotolerance in Arabidopsis thaliana (CLARKE et al., 2009). A foliar spray of lower concentrations of SA conferred heat tolerance to mustard. Further this treatment accompanied with hardening at 45 °C for 1 h enhanced the H<sub>2</sub>O<sub>2</sub> level and this also reduced the CAT activity, thereby increasing the potential of plants to withstand the heat stress (DAT et al., 1998). A similar response was observed in potato plantlets, raised from the cultures, supplemented with lower concentrations of acetyl salicylic acid (LOPEZ-DELGADO et al., 1998). LARKINDALE and HUANG (2004) pointed out the enhanced heat tolerance in plants of Agrostis stolonifera, pre-treated with SA enhanced the protection of plants from oxidative damage. These authors further reported that the pre-treatment with SA had no effect on POX activity, whereas, the CAT activity was shown to decline as, compared to control. Moreover, the treatment enhanced the activity of enzyme ascorbate peroxidase. Contrary to this, an enhanced activity of CAT and SOD was observed in heat stressed plants of Poa pratensis, after the treatment with SA (HE et al., 2005). In a study carried out by CHAKRA-BORTY and TONGDEN (2005) and (Tab. 1), it was reported that the application of SA in Cicer arietinum reduced heat stress induced membrane injury and enhanced the protein and proline contents significantly with a concomitant induction of various stress enzymes viz. POX and APX.

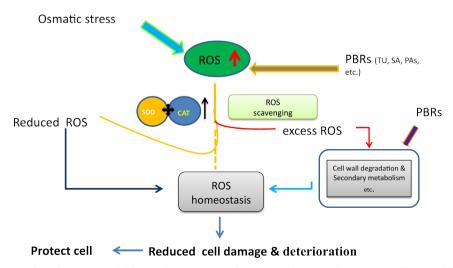


Fig. 2: Cellular level representation of scavenging ROS (reactive oxygen species) induced by plant bio-regulators (PBR like TU: thiourea; SA: salicyclic acid; PAs: Polyamines); through enhancing the antioxidative system (e.g. SOD: superoxide dismutase; CAT: catalase), maintains redox homeostasis and reduced cell damage and deterioration.

# Knowledge gaps carrying forward scientific leads from lab to farmer's field

The use of PBRs is a unique facet of biotechnology and a new approach of manipulating plant biochemistry for enhancing productivity and quality. Although, there is a wide range of PBRs that are commonly used to improve plant growth, development, defense and productivity, the molecular mechanisms of their effects still remain to be fully elucidated. In addition, their commercialization depends upon several factors such as their stability under the field conditions, inertness, cost-effectiveness, ease of application and versatility towards different stresses. From the practical point of view, both endogenous and exogenous substances can be regarded as PBRs if they exert an influence on the growth and development of the plant, in low concentrations and without having any biocidal or nutritive action. Since the environmental conditions before, during and after application of PBR may influence its effect on a given plant, it is critical to develop specific protocols for each PBR before it can widely be commercialized. In fact, changing environmental conditions in the field causing fluctuations of multiple parameters simultaneously such as temperature, humidity makes it difficult to utilize the results obtained from controlled experiments into practical applications for growers. In addition, plants face multiple stresses under farmer's field conditions and combinations of PGRs may be needed to achieve significant impact on growth and productivity. Another aspect which needs to be thoroughly explored is the economic feasibility of commercializing thePBRs from the point of view of agrochemical industry and the customers/users which are the smallholder farmers.

# Plant growth regulator as integral part of management of crop plants under stress

Under stressful conditions, crop management is a key component for optimizing inputs and reducing yield losses. Plant growth regulators play a vital role in determining growth, development and productivity of crops. Advances in ABA signaling science have already allowed refined development of crop management techniques for reducing irrigation input (WILKINSON et al., 2012). Ethylene physiology is accessible to manipulation via crop management. Syngenta and -Dow agosinces have developed a new crop management technique to reduce crop ethylene perception and reduce stress-induced grain yield losses, based on novel applications of 1-MCP (1- Methylcyclopropene). Liquid foliar applications of 'Invinsa' (AgroFresh Inc., USA), which produces gaseous 1-MCP, can act on a range of ethylene-associated process. Crop management using plant-growthpromoting rhizobacteria (PGPRs), as either seed treatments or soil additions, that reduce plant ethylene accumulation under stress by metabolizing the ethylene precursor at the root-soil interface, also increase yields of legumes in the field (BELIMOV et al., 2009). The extent of stress-ethylene production may be genotype-dependent (BALOTA et al., 2004). This provides scope for genetic or management modulation of water use and carbon gain in field crops.

## Quality control and biosafety aspects for impending bioregulatory technologies

While working with any PBR, the first step is to optimize its exact dose and mode of application. Initially, an optimum dose can be derived either from pot based experiment or pilot scale field data and then the same can be tested using multi-location field trials in different agro-climatic zones for at least three calendar years. During these trials, various modes of PBR application such as seed soaking, soil applied and foliar spray or combination of these can be optimized for maximum crop yield with minimum input of PBRs. Once, the exact dose and mode of application is optimized, there are many quality controls (QC) and biosafety aspects which need to

be addressed before recommending any PBR for field application. The first QC is to check whether the PBR application can lead to any toxicity in human or other animals. Since, most of the PBRs are chemical based, it is difficult to say that they are completely safe for human consumption. This is because the term "safe" can be explained only with respect to particular dosage. Thus, at least, the dose at which any PBR is being applied onto the field should be non-toxic. This can be explained in a much better way by taking the example of thiourea which is the most widely used PBR for different crops. The IARC (International Agency for Research in Cancer) has categorized thiourea in Group-3 which states that the evidence of carcinogenicity is inadequate and is limited to animal testing and hence, is not classifiable as probable carcinogen for humans. On the basis of animal testing, "lowest-observed-adverse-effect-level" (LOAEL) and "no-observed-adverse-effect-level" (NOAEL) are derived as 27.5 and 6.88 mg thiourea per kg body weight per day, respectively. The concentration of thio-urea which is normally used for field studies is in the range of 5-10 mM (approximately 250 g/hectare). Since, the foliar application is preferred mode of application, we can assume  $\sim$ 50% uptake of thiourea by plants. With this estimate, one hectare of land having ~1,000,000 wheat plant (standard recommendation from Department of agriculture, Queensland University; http:// www.daff.qld.gov.au) will have ~0.25 mg of thiourea/plant. This is the maximum carry over concentration which is more than 2000fold less than the NAOEL limit. Additionally, thiourea degradation through plant peroxidases will further reduce its residual concentration. Thus, taking these calculations into account, field application of thiourea in the range of 5-10 mM can be considered as safe. Towards this endeavor, yet another important aspect is accumulation potential of thiourea in soil and air. Owing to its very low vapour pressure, the significant adsorption of thiourea on airborne particles is not expected. Due to its solubility in water (137 g/litre at 20 °C), the washout from the atmosphere by wet deposition (fog, rain, snow) is assumed to be significant. From water solubility and vapour pressure data, a Henry's law constant in the range of  $5.58 \times 10^{-9}$  to  $8.44 \times 10^{-9}$  Pa. m3/mol can be calculated, indicating that thiourea is not expected to volatilize from aqueous solutions. Based on these data, the hydrosphere is expected to be the main target compartment for this compound. In hydrosphere, thiourea did not undergo any significant ion exchange or other sorption process and remains in the soil solution as neutral thiourea. Although, thiourea is hydrolytically stable and from UV spectrum it also appears that direct photolysis of thiourea in air or water is not expected. However, thio-urea may get photo-oxidized by hydroxyl radicals with a half-life of 2.4 h. In hydrosphere, the specific rate constants for the reaction of thiourea with hydrated electrons and hydroxyl radicals are given as  $3.0 \times 10^{+9}$ /mol per second (pH 6.4) and  $4.7 \times 10^{+9}$ /mol per second (pH 7). Based on hydroxyl radical concentration of  $1 \times 10^{-16}$  mol/ litre in water, a half-life of 17 days can be calculated which is again depended upon the nature of soil microorganism. Cultures of different fungi isolated from soil behave differently with respect to thiourea degradation. For instance, Aspergillus glaucus, Penicillium citrinum and Trichoderma viride took up around 30-50% of initial thiourea concentration at 0.1 g/litre even after long incubation periods of 46 to 106 days and converted not more than 15-17% of thiourea sulfur to sulfate; however, the same concentration was completely removed within 7 days by Penicillium rugulosum. Based on these data on soil sorption, biodegradation in soil and calculated Koc value; accumulation of thiourea in geosphere is less likely and hence, thiourea based PBRs can be assumed to cause minimum damage to soil ecosystem. Apart from human toxicity, residual grain concentration and ecological imbalance, there is yet another QC which is most important before any PBR is released for field application. This is to evaluate the effect of PBR application on grain nutritional composition. Thus, along with the yield, almost all the field trials with any PBR

should be accompanied with the analysis of grain nutritional profile and only after confirming the minimal change, their application should be recommended. In recent years, although a significant progress in PBR based research has been seen but, most of these are limited with either yield data or underlying molecular mechanism. For most of the PBRs, information about human toxicity, residual grain concentration, nutritional imbalance and ecological bio-safety are not available and hence, they did not get the approval to be used in farmer's field. Thus, there is a great need to give equal attention to these issues so as to increase the chance of converting more and more PBR based research into actual technology.

#### Way forward

Ample of research investigations have been carried out to estimate efficacy of the PBRs under drought, saline and high temperature conditions in crops. Many of such studies depended on pot culture or single location or season experiment. Often scientific leads were not carried forward for large scale evaluation and validation in field conditions, possibly for several reasons including bio-safety measures that may have adverse impact on human health, soil quality and eco-friendly usage. Further, this could also be attributed to lack of information on effect of residues in grain and soil that can impact on human health and ecosystem of which soil microbes especially on *rhizo-bacteria* are the useful and vulnerable component. Hence, there is need for bio-safety, nutritional imbalance, non-residual deliverable product and cost effective PBR based formulation and technology that can address the alleviation of abiotic stresses.

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