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Review

24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants

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ABSTRACT

Drought stress is one of most dramatic abiotic stresses, reduces crop yield significantly. Application of hormones proved as an effective drought stress ameliorating approach. 24-Epibrassinolide (EBL), an active by-product from brassinolide biosynthesis increases drought stress tolerance in plants significantly. EBL application enhances plant growth and development under drought stress by acting as signalling compound in different physiological processes. This article discussed potential role of 24-epibrassinolide application and drought tolerance in plants. Briefly, EBL sustains or improves plant growth and yield by enhancing carbon assimilation rate, maintaining a balance between ROS and antioxidants and also plays important role in solute accumulation and water relations. Furthermore, we also compared different EBL application methods and concluded that seed priming and foliar application are more productive as compared with root application method. In conclusion, EBL is very impressive phyto-hormone, which can ameliorate drought stress induced detrimental effects in plants.

1. Introduction

Drought is one of most unpredicted and uncontrolled environmental setback, posing several devastating effects on plants (Golldack et al., 2014; Anjum et al., 2017a). Among all the other abiotic stresses, drought stress is considered one of the serious threats to crop production under arid and semiarid regions of the world limiting plant growth and productivity (Anjum et al. 2016a, 2017b; Hussain et al., 2018a). Drought stress induces detrimental effects on crop productivity by arresting numerous plant metabolic processes such as loss of turgor, carbon assimilation rate, leaf gas exchange and increased oxidative damage thereby leading to crop failure (Farooq et al., 2009a; Hussain et al., 2018a). Plant responses to drought stress are very complex and depend on several factors such as severity and duration of drought stress and, growth stage of plant, genetic potential of the plant and environmental factors (Zhu, 2002; Jaleel et al., 2009; Anjum et al., 2016b). Drought stress limits enzymatic activity, leaf development, disruption of ion absorption, and ultimately causes losses in crop productivity, are sum of prominent effects of drought stress (Farooq et al., 2009b; Anjum et al., 2017a; Todaka et al., 2017).

Several agronomic and physiological practices are employed to mitigate the adverse effects of drought stress and to induce drought stress tolerance in plants. Application of plant hormones is one of promising and practical strategies to enhance crop productivity under stress conditions (Anjum et al., 2016b; Chen et al., 2018). There are evidences, showing exogenously applied growth regulators can improve tolerance in plants to different abiotic stresses such as drought, heavy metal stress as well as salt stress (Anjum et al., 2016a,b; Shahzad et al., 2018; Tanveer et al., 2018). Brassinolides (BLs) are a new class of phytohormones, which play multiple roles in plant growth and

Abbreviations: 24-eppibrassinolide, EBL; Brassinolides, BL; Photosynthesis, Pn; Evapotranspiration, Et; stomatal conductance, Gs; Intercellular CO_2, Ci; Rubisco activase, RCA; non-photochemical quenching, NPQ; ribulose-1, 5-bisphosphate carboxylase/oxygenase, Rubisco; phosphoenol pyruvate carboxylase, PEPCase; NADP-malic enzyme, NADP-ME; fructose-1, 6-bisphosphatase, FBPase; pyruvate orthophosphate dikinase, PPDK; maximum fluorescence in dark-adapted leaves, Fm; minimum fluorescence in dark-adapted leaves, F0; the quantum yield of PS2, Φ_{PSII}; Photochemical quenching coefficient, q_p; Electron transport rate, ETR; Relative energy excess at the PSII level, EXC; Malondialdehyde, MDA; Superoxide dismutase, SOD; Catalase, CAT; Peroxidase, POD; Ascorbate peroxidase, APX; Glutathione reductase, GR; Ascorbic acid, ASC; Monodehydroascorbate reductase, MDHAR; Dehydroascorbate reductase, DHAR; GR Glutathione reductase, GR; 3-KETOACYL-COA SYNTHASE 9, KCS9; Glycosylphosphatidyl inositol anchored lipid protein transfergene 2, LTPG2; Gdsl-like lipase fatty acid reductase 1, FAR1; Fata acyl-acp thioesterase 1, FATA1; fatty acid elongation 1, FAE1

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development (Kim et al., 2009). 24-epibrassinolide (EBL) an active by-product from brassinolide biosynthesis has ability to stimulate different plant metabolic processes such as photosynthesis (Sairam, 1994), protein and nucleic acid biosynthesis (Bajguz, 2000). In plants, EBL is proposed to be biosynthesized via two pathways included compestanol dependent or compestanal independent pathway however its exact mechanism is unknown (Tanveer et al., 2018). EBL increases the activity of ATPase, and carbon dioxide fixation in maize (*Zea mays* L.), activities of phosphoenol-pyruvate carboxylase (PEPcase) and ribulose-1,5-bisphosphate carboxylase (RuBPase) and concentration of soluble protein in wheat (Braun and Wild, 1984). Apart from its role in normal plant growth and development, EBL has anti-stress effects on plants helping to mitigate the adversities of different abiotic stresses including drought, cold, salt and heavy metal stress (Janeczko et al., 2005; Bajguz and Hayat, 2009; Anjum et al., 2011; Shahzad et al., 2018). Several studies indicated EBL induced growth enhancement under drought stress however little information is available on mechanism conferring EBL induced drought stress amelioration. In the following sections, effects of EBL application on physiological growth of plants have been discussed. Moreover different application methods have also been compared and discussed.

2. Signalling pathway linking EBL signals and plant biological processes under drought stress

24-Epibrassinolide is an active by-product, produced during brassinolide biosynthesis, however molecular identification of 24-Epibrassinolide biosynthesis pathways has yet to be discovered (Tanveer et al., 2018). It has been suggested that EBL might be produced from brassinolide or castasterone (a substrate for brassinolide) nonetheless it is also unknown how EBL would be generated from these two substrates. However it was suggested that EBL is capable of activating BR signalling in plants (Schmidt et al., 1997; Xi and Yu, 2010). Though studies have showed that exogenous application of EBL can improve different abiotic stress tolerance in plant by acting in different ways (Shahzad et al., 2018; Sharma et al., 2018; Tanveer et al., 2018), however it is still unclear how EBL signals different plant defense systems to activate under stress conditions. Therefore future research is required to further explore the involvement of EBL in different metabolic pathways.

In plants, brassinosteroids (BRs), that include 24-epibrassinolide (EBL), are sensed by BRASSINOSTEROID-INSENSITIVE 1 (BR1) receptor kinase and activate distinct signal transduction cascade and cellular responses (Clouse, 2011) This kinase is located on plasma membrane and has been suggested dual-specific in action (Oh et al., 2009). Moreover two sub domains of BR1 receptors, include leucine-rich repeat and serine/threonine kinase receptor, are necessary for the effective transmission of signals (Taiz and Zeiger, 2006). Under stress conditions, BRI1 receptor becomes active after binding to BL, due to its improved autophosphorylation and association with a second membrane localized receptor (BR1-associated receptor kinase 1) BAK1 (Cano-Delgado et al., 2004). Moreover, BAK1 probably acts as a co-receptor with BRI1 and can positively regulate BRI1 function due to physical interactions and transphosphorylation (Chinchilla et al., 2009). Nonetheless it is still unknown about the main role of BAK1 is either to activate the BR receptor or to promote receptor endocytosis (Vert, 2009). Contrarily, Wang et al. (2007) showed that BAK1 was able to affect BR signalling in rice. In following subheadings; we have discussed role of EBL as signalling compound in different processes-based on available literature.

2.1. EBL signals cell division

It has been reported in different studies that EBL improves yield by improving plant growth and development, nonetheless exact molecular mechanism is not known. In a study, it has been suggested that BLs play a key role in *Arabidopsis* cell division in mutant *det2* (*de-etiolated2*) suspension cultures, where it was shown that EBL caused an increase in transcript levels of the gene encoding cyclin-D3, a regulatory protein of the cell cycle. Cyclin-D3 is also regulated by cytokinins, and it may be significant that EBL can efficiently substitute for zeatin (a naturally occurring cytokinin) in the growth of *Arabidopsis* callus and suspension cultures (Hu et al., 2000). Moreover, EBL mediated high photosynthesis activity also results in high production of photo-assimilates, which further increases yield under drought stress. Moreover, EBL may also regulate the activity and biosynthesis of cell wall modifying enzymes and other proteins such as xyloglucan endotransglucosylase/hydrolase, cellulose synthase, sucrose synthase and glucanases, thereby regulating cell elongation.

In a study, the dwarf nature of BR-deficient mutants and the ability to return to normal phenotype with the application of BRs shows the key role of BRs in plant growth and development (Wang et al., 2007). Evaluation of cell orientation in the wild-type *Arabidopsis* as well as BR mutants *cbb*, *dwf4*, *cpd* and *dim* using light- or electron-microscopy indicated that longitudinal cell expansion was markedly impaired in the BR mutants (Altmann, 1999; Sakurai, 1999). The overexpression of *dwf4* gene, which encodes an enzyme responsible for regulating a putative rate-limiting step in BR biosynthesis, has been shown to promote hypocotyl length in *Arabidopsis* (Choe et al., 2001). Moreover, role of BRs has also been demonstrated that they may promote cell division and elongation by regulating the transport of water via aquaporins as well as regulating the activity of a vacuolar H^+-ATPase subunit (Friedrichsen and Chory, 2001; Morillon et al., 2001).

2.2. EBL interacts with hormones

EBL may interact with different other endogenous hormones under stress conditions to improve plant growth and yield. Seed germination is one of most vulnerable stage to drought stress in plant (Anjum et al., 2017a). BRs promote seed germination by counteracting the inhibitory effect of ABA and regulate plant reproductive development, thus affecting seed yield. This is because BR has been suggested to act in parallel with GA to promote cell elongation and germination (Leubner-Metzger, 2001). In another study, it has been seen that, EBL can substitute cytokinin. This is suggesting that EBL may interact with different enzymes to trigger cell proliferation, growth improvement and yield formation under stress environment. It has been shown that *MOTHER OF FT AND TFL1* (*MFT*) responds to both ABA and GA signals to regulate seed germination (Xi et al., 2010). Moreover in another study it was shown that in *mft* deficient mutants, BL application did not fully antagonize the inhibitory effects of exogenous ABA on seed germination, suggesting that BR promotes seed germination against ABA partly through *MFT* (Xu and Yu, 2010).

Similarly, EBL also has been found as substitute for cytokinin during cell division. Shakirova et al. (2002) noted that EBL application can increase endogenous cytokinin level by 2 folds. Moreover it was seen that EBL upregulated the transcription of the CycD3, a D-type plant cyclin gene through which cytokinin activates cell division (Hu et al., 2000). However this response was endogenous EBL level, as Oh and Clouse (1998) reported that high EBL concentration is less effective or inhibitory to cell division, thus dose dependent relationship should be examined in future to further explore EBL-cytokinin pathway. Recently, Yuldashev et al. (2012) further examined the mechanism behind EBL and cytokinin interaction; found that Elevated cytokinin level has been maintained only in the presence of EBL and this was associated with EBL induced high activity of cytokinin *O*-glucosides. Moreover they also noted that EBL also reduces the expression of genes for cytokinin oxidase production, an enzymes responsible for cytokinin degradation.

2.3. EBL interact with lipid metabolism

It is well known that under stress conditions, lipid metabolism is

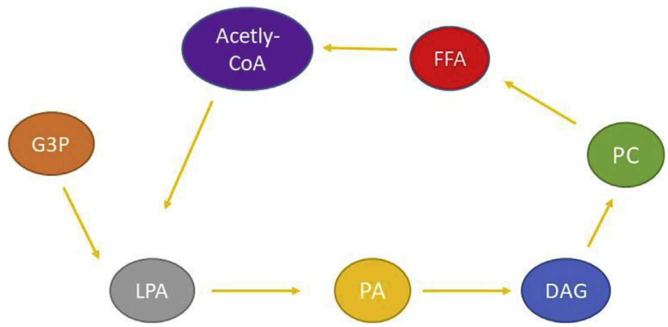

Fig. 1. Simplified scheme of plant TAG biosynthesis pathways (Pokotylo et al., 2014). DAG, diacylglycerol; G3P, glycerol 3-phosphate; FFA, free fatty acids; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine.

affected drastically and excessive ROS production results in high lipid peroxidation. EBL has been found as an effective phyto-hormone, which activates antioxidant system (on one hand) and protect and improve lipid metabolism in cells (on other hand). Moreover, intracellular role of BL may be associated with phospholipases that control many plant growth reactions (Wang et al., 2012). A simplified schematic presentation of lipid metabolism has been presented in Fig. 1. Phospholipases hydrolyse membrane lipids to produce second messengers, which are essential for regulatory signalling. Moreover, phospholipases also contribute to generic lipid metabolism and lipid turnover (Van Meer et al., 2008). Peng et al. (2010) showed that overexpression of phospholipase Dα resulted in improved drought tolerance. EBL has been found as regulator of phospholipases and increases the production of phosphatidic acid (Pokotylo et al., 2014). Moreover it has also been shown that BL application also activated the accumulation of diacylglycerol in tobacco BY-2 cells (Wimalasekera et al., 2010; Pokotylo et al., 2013). This was suggested due to increase in the activity of non-specific phospholipase C and/or phospholipase D enzymes that catalyse the hydrolysis of phosphatidylcholine into diacylglycerol and phosphatidic acid, respectively (Hong et al., 2008; Kocourková et al., 2011). Moreover it has also been seen that EBL reduces the production of *n*-butyl (an inhibitor of phospholipase D) and increases phosphatidic acid production (Pokotylo et al., 2014). In drought it has not been studied that which genes relating to lipid metabolism are EBL responsive however under salt stress it has been identified that genes such as KCS9, LTPG2, FAR1, FATA1 and FAE1 were upregulated by BL, thus showing EBL may improve stress tolerance in plant partially by improving lipid metabolism in plants (Pokotylo et al., 2014).

3. 24-Epibrassinolide application and plant responses under drought stress

3.1. Plant yield response under drought stress

Since drought stress in plants negatively affects their growth and development via enhanced production of ROS, reduced photosynthesis, disturbance in water balance etc. All these factors ultimately lead to the reduction in the growth and yield of crop plants (Talaat et al., 2015). According to Anjum et al. (2016a), drought stress reduced wheat grain yield by 58% due to reduction in spike length (31%), number of grains per spike (205%), and 100 grain weight (15%). Similarly, Anjum et al. (2017b) suggested that drought induced reduction in maize gain yield was due to reduced photosynthetic activity with subsequent reduced production of carbohydrates and other sugars. Other studies also reported substantial yield reduction in different crop plants such as rice (64% reduction- Venuprasad et al., 2007), chickpea (upto 66% reduction- Mafakheri et al., 2010), and millet (43% reduction- Seghatoleslami et al., 2008) and sunflower (up to 83% reduction- Nezami et al., 2007).

Exogenous application of 24-Epibrassinolide (EBL) results in recovery of growth and yield of plants under drought stress, thus improves crop yield (Yu et al., 2004; Anjum et al., 2011). Talaat et al. (2015) reported a significant improvement in the yield of maize plants after exogenous application of EBL under drought stress. They noticed an enhancement in grain number per plant as well in grain yield per plant after EBL treatment. Anjum et al. (2011) have also reported a significant recovery in the yield of maize in EBL treated plants, which were grown under drought stress. They noticed a significant increase in number of kernals per cob, biological yield per plant and grain yield per plant after EBL application under drought stress. In *Vigna radiata*, recovery in the yield (number of pods/plant, number of grains/pod and grain yield/plant) was also observed after exogenous brassinolide treatment of plants grown under drought stress (Lal et al., 2013). EBL application recovered number of leaves, number of flower clusters, lycopene content, fruit diameter and fruit yield in tomato plants subjected to drought stress (Jangid and Dwivedi, 2017). Xiong et al. (2016) also observed enhancement in plant height and yield of grass pea plants after exogenous application of EBL under drought stress. Studies also suggested that increase in yield due to EBL application is also associated with improved photosynthetic efficiency of plants under drought stress (Anjum et al., 2011; Lima and Lobato, 2017). Ali and Ashraf (2008) suggested that total grain yield was mainly increased by increase in grain size which might have been due to EBL induced increase in higher photosynthesis and translocation of more photo-assimilates towards grain. Similarly, in tomato, Goetz et al. (2000) found that exogenous application of BRs caused enhancement of cell-wall-bound invertase activity with a concurrent increase in sucrose uptake. Furthermore, they also found tissue-specific induction of mRNA for extra-cellular invertase.

From these findings it can be suggested that EBL-induced increase in growth and grain yield may have been due to more supply of carbohydrates through activation of appropriate enzymes. Moreover, EBL induced growth improvement could be associated with the role of BLs in cell division under stress environment. In a study it was shown that transformed rice plants with BAK1 produced more grains and this was due to better grain filling rate and leaf development as compared with untransformed plants (Khew et al., 2015). In conclusion, it is well known, so far, EBL improves plant growth and yield under drought stress however exact mechanism relating to EBL induced high carbon assimilation and yield improvement has yet to be validated. Therefore future research is required to further examine this relationship.

3.2. Light harvesting and photosynthesis process under drought stress

Drought stress also limits carbon fixation process by decreasing the activities of numerous important enzymes such as Rubisco, PEPCase, PPDK and EBPase (Farooq et al., 2009). Brassinolides can improve photosynthesis under drought stress by maintaining positive turgor potential, enhanced leaf area, and better gas exchange (Li et al., 2012b).

EBL application can enhance the light harvesting and carbon-fixation process under drought stress by improving chlorophyll contents, chlorophyll fluorescence and also by triggering the activities of enzymes involved in photosynthesis. Lima and Lobato (2017) reported that EBL application increased chlorophyll *a*, chlorophyll *b* and total chlorophyll contents by 26%, 58% and 33% under drought stress. Moreover, EBL also improves leaf gas exchange and improves CO_2 fixation process under drought stress. Under drought stress, exogenous applied BLs significantly recovered the gas exchange parameters (Anjum et al., 2011). Enhancement in the Pn, Et and Gs after EBL application under drought stress was due to the enhanced efficiency of PSII as well as due to increased carbon fixation process (Lima and Lobato, 2017). Hu et al. (2013) also observed that exogenous application of EBL recovers the photosynthetic performance of *Capsicum annum* by improving the Pn, Et and Gs under drought stress. EBL application also results in reduction of intercellular CO_2 under drought stress which

Table 1
Role of EBL in regulation of the activities of antioxidative enzymes in plants under drought stress conditions.

Plant species	EBL concentration applied	Effect of EBL on the activities of antioxidant enzymes	Reference
Robinia pseudoacacia	$0.2\ mg\ l^{-1}$	Increase in the activities of SOD (25%), CAT (38%) and POD (33%)	Li et al. (2008)
Oryza sativa	0.01 µm	Increase in the activities of SOD (33%), CAT (32%) and APX (27)	Farooq et al. (2009)
Lycopersicum esculentum	1 µm	Increase in the activities of CAT (62%)	Behnamnia et al. (2009)
Zea mays	$0.1\ mg\ l^{-1}$	Increase in the activities of SOD (43%), CAT (32%), APX, (43%), and GR (43%)	Talaat et al. (2015)
Raphanus sativus	0.2 µm	Increase in the activities of SOD (50%), CAT (35%) and APX (34%)	Mahesh et al. (2013)
Cajanus cajan	2 µm	Increase in the activities of SOD (10%), CAT (19%), APX (19%), and AsA (16%)	Shahana et al. (2015)
Gossypium barbadense	10^{-7} M	Increase in the activities of SOD (50%), CAT (32%) and POD (32%)	Ahmed et al. (2017)
Phaseolus vulgaris	$0.1\ mg\ l^{-1}$	Increase in the activities of POD (13%), AsA (22%)	Younesian et al. (2017)
Triticum aestivum	$0.1\ mg\ l^{-1}$	Increase in the activities of SOD (16%), CAT (60%) and POD (25%)	Zhao et al. (2017)

is correlated with enhanced Pn, hence proposing that EBL may trigger the Rubisco activity which may enhance the assimilation of intercellular CO_2 (Yu et al., 2004).

Though molecular responses of plant to EBL under drought stress has not been examined yet however studies examined biochemical and physiological role of EBL in improving photosynthesis showed that EBL primarily improves photosynthesis process by reducing the negative effects of drought stress on chlorophyll fluorescence and associated traits. EBL improves light harvesting by improving the chlorophyll fluorescence and performance of PSII (Maxwell and Johnson, 2000). Moreover, exogenous applied EBL showed significant effect on Fm, Φ_{PSII}, ETR and q_p and increased these traits by 32%, 74%, 72% and 112% respectively under drought stress (Lima and Lobato, 2017). Nonetheless, Lima and Lobato (2017) also noted an interesting response of F0, showed a decline in F0 values in response to EBL under drought stress. Exact reason cannot be explained due to insufficient published literature however Lima and Lobato (2017) suggested EBL induced F0 reduction might be due to the enhanced flow of photons to the PSII (reaction center) from collector system (Baker and Rosenqvist, 2004). This can also be speculated that EBL may signal the reaction center to maximize the flow of photons and with concomitant higher energy absorption of photons at reaction center, thus improving Fm value as compared with F0 value. This can also be supported by Buonasera et al. (2011) that improved light quenching under drought stress is related to increased flow of energy for the excitation of electrons accepted by plastoquinone and plant showed higher Φ_{PSII}, ETR and q_p. Moreover, Thussagunpanit et al. (2015) studied the action mechanisms of EBL in *Oryza sativa* plants and reported a significant increase in Φ_{PSII} after EBL application. Research conducted by Li et al. (2015) showed that EBL increases the proportion of open PSII reaction centers, improving the efficiency of the capture of light energy for the electron transport chain.

Another positive effect of EBL during photosynthesis is that it reduces NPQ and ETR/Pn during drought stress (Lima and Lobato, 2017). High light harvesting capacity of PSII after EBL application results in reduction of NPQ as well as EXC values (Silva et al., 2012). This can be suggested that EBL improves photosynthesis by reducing over-excitation of election and by limiting non-photochemical quenching at reaction center. Less quenching may also be due to the photorespiration and photoreduction in response to EBL application (Silva et al., 2011; Barbosa et al., 2014). Over excitation of electron can also assist in inducing reactive oxygen species by oxidizing oxygen, thus causes oxidative damage to plants (Flexas et al., 1999; Singh and Reddy, 2011). Rubisco activity increases after the EBL application under drought stress (Zhao et al., 2017). Rubisco activase (RCA) is involved in keeping RUBISCO in active form. There are three subunits of RCA (38–39 kDa, 41–42 kDa, 45–46 kDa). Stability of RCA structure depends upon the interaction between different subunits, resulting in maintaining the initial Rubisco activity under stressful environment in plants (Wang et al., 2010; Chen et al., 2015). Zhao et al. (2017) reported that under drought stress, abundance of 38–39 kDa subunit of RCA was reduced, which was later recovered after the EBL application, resulted in enhanced Rubisco activity. These researchers suggested that EBL enhances initial activity of Rubisco by enhancing the expression of 38–39 kDa subunit of RCA under drought stress. Moreover, they noticed that EBL increased the photosynthetic performance of plants grown under drought stress, possibly by post-transcriptional regulation. Redox homeostasis is also involved in the EBL-mediated increase in RCA expression (Zhao et al., 2017). Furthermore, it has also been seen that EBL also interact with NO and H_2O_2, which play significant role in plant abiotic stress resistance (Cui et al., 2011).

3.3. Redox homeostasis in plants under drought stress

Drought stress disturbs the balance between ROS accumulation and antioxidative defense system of plants leading to oxidative stress and ultimately negatively affects the crop yield (Mittler, 2002; DaCosta and Huang, 2007). These ROS generally include superoxide anions, hydroxyl ions and hydrogen peroxide, which cause lipid peroxidation and electrolyte leakage, resulting in the disturbance of normal cell function (Talaat et al., 2015; Tanveer and Shabala, 2018). To cope-up with the oxidative stress and enhancing the scavenging of these harmful ROS, the internal antioxidative system of plants gets activated (Sharma et al., 2017). However, the enhanced activities of antioxidative enzymes alone are insufficient to protect plant cells from negative effects of ROS generated under extreme drought stress (Talaat et al., 2015). Role of BLs have been well documented in enhancing the scavenging of ROS produced due to the various abiotic stress conditions including drought (Lima and Lobato, 2017; Shahzad et al., 2018; Sharma et al., 2018; Tanveer et al., 2018).

EBL, one of the most bioactive types of BLs, plays a crucial role in ROS scavenging and redox regulation in plants grown under drought stress (Talaat et al., 2015; Lima and Lobato, 2017). However, EBL induced antioxidant production or high antioxidant activity depends on EBL concentration and plant species (Table 1). In cow pea, Lima and Lobato (2017) reported that exogenous EBL application under drought stress significantly enhanced the scavenging of ROS, accompanied by the enhanced activities of antioxidative enzymes (SOD, CAT, APX and POD). This reduction in oxidative stress alleviated the negative effects of drought stress on PSII. Additionally, EBL application reduces EXL, hence revealing a decrease in O_2 photo-reduction. Moreover, SOD activity also gets enhanced after EBL application, playing its role in ROS reduction (Lima and Lobato, 2017). Shahana et al. (2015) noted that lipid peroxidation and H_2O_2 production was reduced by 31% and 42% due to EBL application in pigeon pea and this was due increase in the production of SOD (10%) and APX (19%). Behnamnia et al. (2009) noted that EBL increased CAT activity by 62% activity in tomato under drought stress. Thus it can be concluded that EBL may improve drought tolerance in plants by triggering ROs scavenging system however the efficacy of such system depends on plant species.

Another cycle involved in the scavenging of ROS is AsA-GSH cycle (Talaat et al., 2015). AsA converts superoxide anions and hydrogen peroxide into MDHA/DHA (De Gara et al., 2000). AsA and DHA concentrations enhance under drought stress but AsA/DHA ratio reduces which could be due to the decrease in DHAR and MDHAR activities under drought stress (Talaat et al., 2015). EBL application recovers AsA/DHA ratio in plants under drought stress. Additionally, EBL also regulates the activities of enzymes involved in AsA-GSH cycle, resulting in maintaining the redox state of ascorbate and hence enhances drought stress tolerance in plants (Talaat et al., 2015). Morales et al. (2014) reported that EBL stimulates the AsA synthesis pathway by increasing the AsA precursor formation. GSH is also involved in scavenging of ROS via AsA-GSH cycle (Foyer and Noctor, 2011). Under drought stress, GSH/GSSG ratio decreases but EBL positively regulates GSH/GSSG ratio in plants grown in drought stress (Talaat et al., 2015). They suggested that some amount of GSH might have involved in maintaining the redox state and ultimately helping in quenching of hydrogen peroxide, which results in boosting up the cellular defense system. Moreover the reduction in the ROS like hydrogen peroxide after EBL application might be due to the BL-mediated regulation of the expression of gene (*RBO*) involved in production of hydrogen peroxide (Sharma et al., 2017). EBL down-regulates the expression of *RBO* under drought stress which may be another reason behind less production of hydrogen peroxide after EBL application (Sharma et al., 2017). Bajguz (2000) suggested that increase in the activities of various enzymatic antioxidants after EBL application is due to the BL-mediated transcription/translation of genes involved in antioxidative defense system.

3.4. Solutes accumulation in plants under drought stress

Plants accumulate numerous organic and inorganic compounds of low molecular weight, termed as osmolyte, which play a vital role in osmotic adjustment under drought stress (Blokhina et al., 2003; Farooq et al., 2009; Talaat et al., 2015; Chen et al., 2018). These osmolytes include amino acids, soluble sugars, proteins, polyamines and some members of non-enzymatic antioxidants (Yancey, 2005). Under drought stress, plants accumulate these osmolytes in cytoplasm as defensive strategy to adjust osmotic potential and water potential of cells (Ottow et al., 2005; Farooq et al., 2009). Plants also produced osmolytes differentially at different developmental stages in order to protect plants from drought-induced desiccation. Nonetheless, under severe drought stress, sensitive plants showed reduced accumulation of osmolytes (Serraj and Sinclair, 2002).

BLs are found to be effective in improving drought tolerance in plant by improving osmoytes accumulation, however osmolytes accumulation varies in different plant species in response to different EBL concentration applied. (Table 2). Among different osmolytes, proline is known for stabilizing sub cellular structures such as proteins and cell membranes, scavenging free radicals and buffering redox potential under stress conditions (Ashraf and Foolad, 2007). It also functions as molecular chaperones protecting the integrity of protein and enhances the activity of different enzymes (Szabados and Savouré, 2010). BL application triggers the synthesis of proline in plant cells under water scarcity (Chen et al., 2018). It is believed that BL-application enhances the proline accumulation by stimulating Δ^1-pyrroline-S-Carboxylate synthase, which is the key enzyme of proline biosynthetic pathway (Sharma et al., 2011). Additionally, BL-regulated proline accumulation also plays an important role in maintaining water contents of plant tissues (Anjum et al., 2011). Although the role of proline in improving drought tolerance in controversial however studies showed that EBL induced high proline accumulation is one of EBL induced drought tolerance mechanisms under drought stress. Li et al. (2012a,b) showed that in *C. bungeana* plants, there was no influence on proline accumulation in response to EBL application under non-stress conditions; however under drought stress EBL significantly increased proline accumulation, thus increases drought tolerance in *C. bungeana* plants. Similar results have been reported by Li et al. (2012a,b), who showed that EBL improves drought tolerance by improving proline biosynthesis and soluble sugar accumulation. According to Younesian et al. (2017), an increase in proline accumulation in response to EBL application resulted in the reduction of membrane damage in plants and so tolerance to drought stress was increased by osmotic adjustment method. Moreover, proline has been considered as source of carbon and nitrogen for rapid recovery from stress and EBL application improves drought tolerance in plant by improving proline accumulation and source of energy for the survival of plants under drought stress (Behnamnia et al., 2009). In another study, it was found that EBL treatment under drought stress increased citruline and ornithine and proline contents, thus suggested that EBL may signal the ornithine pathway involving citrulline and ornithine which can participate in the proline biosynthesis (Pustovoitova et al., 2001).

Phenolic compounds are involved in the protection of plants under abiotic stresses by protecting non-photosynthetic membranes from harmful ROS (Blokhina et al., 2003). Soluble phenolics are also enhanced under drought stress and further increase in phenolic compounds take place in plant cells after EBL application (Farooq et al., 2009). Sugars are also one of the important osmolytes involved in protection of plant cells from drought stress and exogenous application of EBL further enhances sugar levels in plants under drought stress, resulting in drought tolerance to plants (Li et al., 2008; Mousavi et al., 2009). Glycine-betaine (GB) in plants under stressful conditions is involved in maintaining membrane structures, decreasing lipid peroxidation, scavenging of free radicals and maintaining cellular structures as well as cellular-redox-potentials (Ashraf and Foolad, 2007). GB also enhances the plant resistance to drought stress and is involved in mechanisms which help in prevention of water loss via osmotic adjustments. Moreover, EBL application also enhances the accumulation of GB (32%) by regulating process of GB-biosynthesis (Talaat et al., 2015). In conclusion, EBL ameliorates drought stress induced negative effects by enhancing osmolytes production and osmoregulation. Moreover EBL induced greater proline and glycinebetaine accumulation in stressed plants also helps subsequently in maintaining membrane integrity, reducing the oxidation of lipid membranes, and in protecting and stabilizing ROS scavenging enzymes.

3.5. Water relation in plants under drought stress

One of the major detrimental effects of drought in plant is relating to significant water loss in tissues and cells (Siddique et al., 2000). EBL helps in recovering the plant growth and development under drought stress by improving the water use efficiency (WUE) and instantaneous

Table 2
Role of EBL in regulation of various metabolites involved in drought stress management in plants.

Plant species	EBL concentration applied	Effect of EBL on the accumulation of metabolites/solutes	Reference
Robinia pseudoacacia	0.2 mg l^{-1}	- Proline (30%) and soluble sugar (55%) contents were increased.	Li et al. (2008)
Brassica napus	10^{-7} M	- Proline (27%) and reduced sugar contents (25%) were increased.	Mousavi et al. (2009)
Oryza sativa	0.01 μm	- Proline were increased by 16%.	Farooq et al. (2009)
Raphanus sativus	0.2 μm	- Proline (37%) and soluble protein (42%) contents were increased.	Mahesh et al. (2013)
Lycopersicum esculentum	1 μm	- Proline and protein contents were increased by 25% and 28% respectively.	Behnamnia et al. (2009)
Zea mays	0.1 mg l^{-1}	- Proline (30%) and glycine-betaine (30%) contents were increased.	Talaat et al. (2015)
Cajanus cajan	2 μm	- Proline and glycine-betaine contents were increased by 12% and 17% respectively.	Shahana et al. (2015)
Phaseolus vulgaris	0.1 mg l^{-1}	- Proline content was increased by 18%.	Younesian et al. (2017)

Table 3
Factors affecting efficacy of exogenously applied 24-epibrassinosteroid (EBL) under drought stress.

Factors affecting EBL efficacy	Effects on EBL application
1. Dose optimization	It can be effective in achieving promising results if optimized concentration of EBL is used.
2. Stage/time of application	Application of EBL at early vegetative growth is more effective than later stages
3. Addition of additives	Addition of additives can increase the penetration and spreading of EBL on leaves or root for better results under stress conditions
4. Duration of treatment	Optimized length of time for soaking seeds in EBL would also be beneficial
5. Mixing fertilizer with EBL	Mixing of fertilizer with EBL can increase the efficiency of both fertilizer and EBL along with reducing labour cost for application
6. Plant cultivar, climatic conditions, type of soil/soil less medium	Cultivar selection, climatic conditions and type of soil are the other factors that can increase or decrease the effectiveness of EBL application in response to drought stress

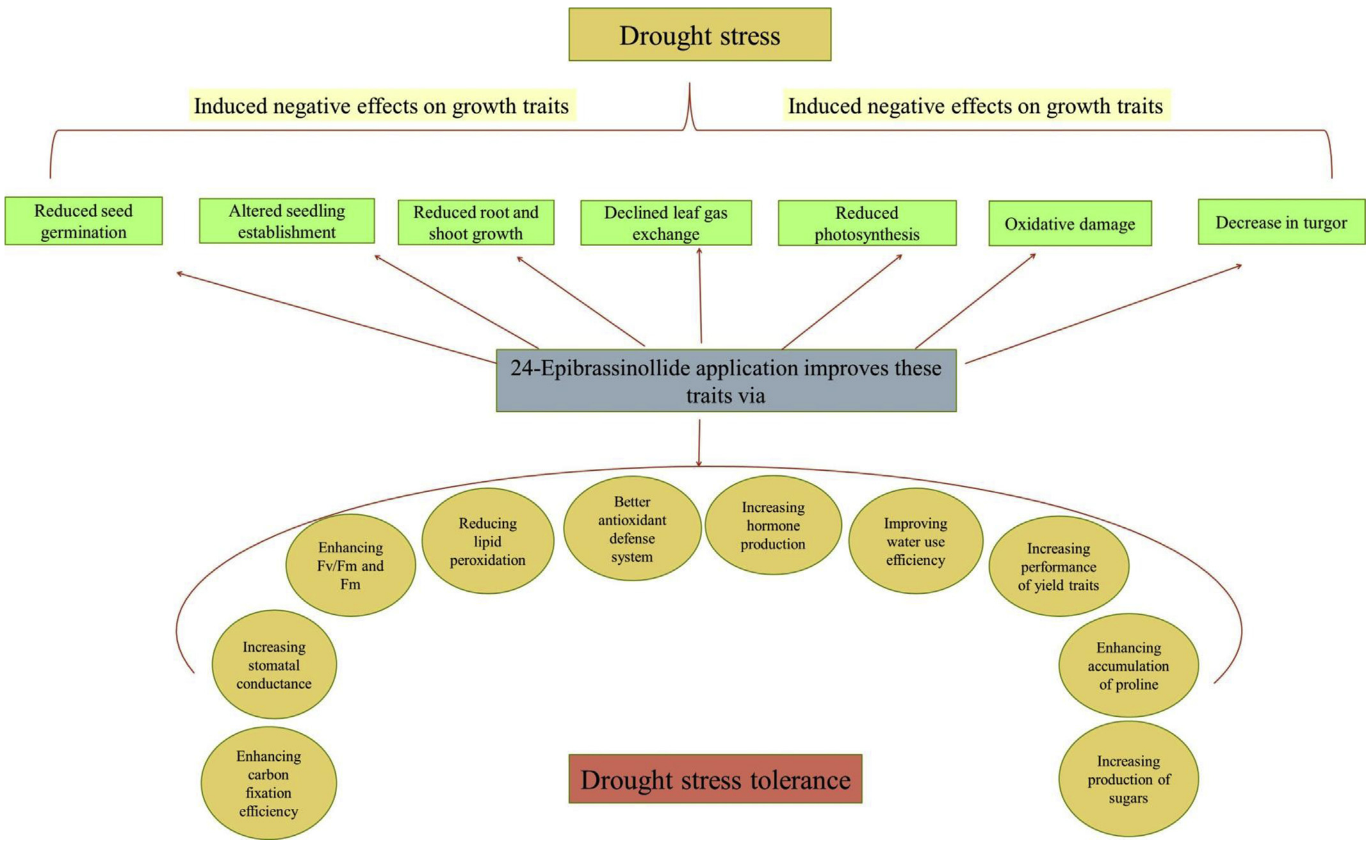

Fig. 2. 24-Eppibrassinolide application improves drought stress tolerance in plants by playing significant role in numerous physiological processes.

water use efficiency (WUEi) (Anjum et al., 2011). EBL also enhances the relative water content (RWC) in drought stressed plants (Li et al., 2012a,b). This recovery in RWC after BR application might be due to the reduced transpiration rate of leaves in drought stressed plants (Li and Feng, 2011). EBL also maintains the RWC in plants under drought stress by improving the water potential, osmotic potential and pressure potential (Farooq et al., 2009). This is due to the involvement of BLs in maintaining the permeability of plasma membrane under drought stress (Hamada, 1986). Lima and Lobato (2017) also reported the recovery in WUE and water potential of *Vigna unguiculata* plants grown under drought stress in response to EBL treatment. Yu et al. (2004) suggested that improvement in water relations due to EBL application under drought could be possibly due to the high accumulation of various osmoprotectant solutes like sugars. In another study, Mousavi et al. (2009) reported that EBL application improves fresh weight of plants under drought stress by improving proline accumulation, thus suggesting that EBL improves water retention in plant tissues by increasing the accumulation of proline. Likewise, according to Talaat et al. (2015) EBL induced high accumulation of proline and glycine betain resulted in less loss of water from plant tissues, thus improves water contents in plants. In another study, Khamsuk et al. (2018) observed that EBL application improved RWC in chili pepper plant up to 71% under drought stress and this was due to high proline accumulation, reduced membrane damage and oxidative stress.

4. Comparison of different EBL application methods

Application of growth regulators such as 24-epibrassinolide (EBL) generates promising results in inducing drought stress tolerance in plants. EBL exogenous application by different techniques such as seed priming, or foliar application or root application can increase the capability of plants to withstand drought stress. Nonetheless, efficiency of different application methods depends on numerous factors. Moreover, a number of researchers have reviewed the potential utilization of EBL in improving growth under different abiotic stresses (Vardhini et al., 2010; Shahzad et al., 2018; Sharma et al., 2018; Tanveer et al., 2018). However it has never been discussed about potential pros and cons of different application methods and their efficiency in improving EBL induced drought stress amelioration. Potentially, there are thee way to exogenously apply EBL, include seed priming, foliage application and root application (Vardhini et al., 2011; Li et al., 2012a,b; Mahesh et al., 2013; Hussain et al., 2018b).

Comparing different application methods in different studies revealed that practically, application of EBL via seed treatment and foliar spray is more convenient than root application. However, efficacy of EBL application highly depends upon the growth stage of plant development and the concentration of EBL used (Table 3). For instance, some studies showed that application of EBL at early growth stage was more effective than at later stage (Ashraf and Foolad, 2007; Zhang et al., 2007; Ali and Ashraf, 2008). Moreover, determination of proper formulation of spraying solution is essential before applying on target plants (Khripach et al., 2003). To increase the efficiency of spraying solution, it must contain some additives that can facilitate spreading and increase the absorption of active substance on the leaf surface (Shahbaz and Ashraf, 2008; Akram et al., 2009). Inclusion of additives helps to delay the dryness of the leaf surface and to ensure the penetration of growth regulator through cell walls. Similarly, effectiveness of pre-sowing treatment not only depends on the concentration of EBL but also the duration of soaking with grow regulator. Therefore, EBL could be applied along with fertilizers and through fertigation under field conditions (Khripach et al., 2000). This application method may prolong the treatment but also can reduce the labor cost required to apply it separately (Khripach et al., 2000). While other factors that need to note are but not limited viz., plant cultivars, existing climatic conditions, soil types and fertilizer levels to be applied (Ashraf et al., 2010). For example, effectiveness of EBL application in rice under field condition significantly differed under different temperatures and light conditions (Kamuro, 1999). Therefore, it is vital to optimize the proper application protocols and EBL concentration for a specific plant species and growing conditions before field application on a larger scale.

5. Conclusion

Application of 24-Epibrassinolide can negate the detrimental effects of drought stress in plants (Fig. 2). Literature shows that EBL is an active brassinolide and acts as signalling compound, which signals different metabolic processes such as cell division or lipid peroxidation in plants. Moreover EBL reduces the production of reactive oxygen species by activating ROS scavenging enzymes. EBL protects ultrastructure of photosynthetic pigment apparatus from degradation thus increases photosynthesis and other leaf gas exchange traits. EBL application increases the accumulation of different compatible solutes especially proline to adjust osmotic status of plant under drought stress. Among different application methods, application of EBL via seed treatment and foliar spray is more convenient than root application. Different factors influences the efficacy of these application methods, therefore further research is required to further examine the efficiency and optimization of these methods.

Author contribution

All authors listed have made a substantial, direct and intellectual a contribution to the work, and approved it for publication.

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