Interactive Role of Brassinosteroids and Calcium Ameliorates in Response to the Aluminium Toxicity in Plants

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ABSTRACT

Aluminum toxicity is considered one of the major growth limiting factors for crop production on acid soils worldwide, and pose a major challenge to agriculture sustainability. At low pH, the most toxic form of Al+3 is released into the soil and causes extensive damage to plants, especially in the root. To develop high tolerance against Al toxicity is the prime concern of plant science. Research has reported that the Brassinosteroids play a diverse role in plant growth, development and stress response. Although the BRs have been exhaustively studied, a comprehensive overview of the manner in which BRs participate in calcium signaling pathways under Al toxicity and regulating plant Al tolerance has not yet been undertaken. In this article, we highlight the interactive role of BRs and Ca, regulating plant growth at the physiological, biological and molecular level, focusing mainly on the BRs induced Ca signaling participate in regulating reactive oxygen species suggesting an elevation in ROS generation confer plant Al resistance. Significantly, this review addresses the current, albeit partially understood, emerging aspects on (i) Al-induced physiological, biochemical, and genotoxic mechanism and responses in plants and (ii) the role of BRs and Ca in the modulation of Al-induced toxicity in plants. These finding might provide further potential for the relevance of BRs and Ca in phytoremediation and Al detoxification in crops.

KEYWORDS: Nicotinamide adenine dinucleotide phosphate hydrogenase

1. INTRODUCTION

Aluminum (Al) is the most abundant and third most common metal in the earth's crust, constituting of about 8% of total soil minerals. Despite being abundance in the soil, Al has no specific biological role for plants life cycle. The total concentration in the soil and speciation of Al depends on the pH of the soil and the chemical environment of the solution (Bojórquez-Quintal *et al.*, 2017). At neutral and alkaline pH Al occurs in the combined form an oxide or even more commonly as aluminosilicates, which are nontoxic to plants. At low pH (about 4.3) trivalent aluminum (Al^{3+}) is the most abundant form and has a maximum impact on plant growth (Bojórquez-Quintal *et al.*, 2017). Surprisingly the impact of Al on plants growth, both beneficial (see Table 1) and toxic depends on the concentration of Al, growth condition, type of the species and duration of exposure to the metal. However, the pH < 5.0 the solubility of Al compounds increased and become phytotoxic impairing the root growth and functions (Matsumoto, 2000). Therefore Al toxicity thought to be the primary limiting factor for plant growth and productivity on the acidic soil in many developing countries (Horst *et al.,* 2010; Kochian *et al.,* 2015; Rengel *et al.*, 2015; Silva *et al.*, 2012).

Ca is an essential structural, metabolic and signalling element. It is needed for structural roles in cell wall and membrane and acts as a counter cation for organic and inorganic anion in the vacuole and intracellular messenger in the cytosol coordinating responses to numerous developmental processes and environmental responses (Bootman, 2012). The concentration of cytoplasmic Ca in plants increases in the response of various stimuli like biotic and abiotic stresses and mediate a specific signal response (Hochmal *et al.*, 2015; Sanders *et al.*, 2002; Steinhorst and Kudla, 2014). The accumulation of Ca is stimulus-specific in term of its amplitude, frequency, and duration in response to a signal. Ca also acts as a plant hormone in the regulation of various cell functions in the plants. It also reduces the toxic effect of heavy metals in the plant by producing reactive oxygen species (Sanders *et al.*, 2002).

BRs are considered as a new class of steroidal plant hormone that is structurally similar to animal and insect hormone. BRs is ubiquitously distributed in the plant kingdom and mostly found in all parts of plants including roots (Tang *et al.*, 2016). Among BRs, brassinolide, 24-epibrassinolide, and 28-homobrassinolide are the three most bioactive and

generally used in physiological studies. This hormone elicit a wide range of physiological and developmental processes in plants like cell division and expansion, stem elongation, root, and pollen tube growth, leaf bending and epinasty (Bajguz and Tretyn, 2003; Choudhary *et al.,* 2012). BRs also involved in the biosynthesis of nucleic acid and proteins, induction of ethylene, activation of enzymes and photosynthesis. Moreover, BRs is also reported to have an ameliorative effect on plants subjected to various biotic and abiotic stresses (Arora *et al.*, 2012; Sharma *et al.*, 2013).

2. Physiological effect of Al toxicity on plants

Aluminum toxicity is one of the most prevalent forms of metal stress-limiting crop production in acid soils in the tropics. It has been reported that 30-40% of the arable soils of the world are acidic. These soils are mainly associated with regions of high rainfall where base cations like Ca^{2+}, Mg^{2+}, K^+, and Na^+ etc. have been leached from the soil and replaced by toxic Al^{3+} cations released from soil mineral weathering. The Al^{3+} cation effectively inhibits root growth and hampers plant development and thereby reduces productivity (Famoso *et al.*, 2010; Mahajan and Tuteja, 2005). Table 2 shows the effects of Al toxicity in the plants.

2.1. Effect of Al on Root Growth

Al primarily affects root growth by interfering with processes decisive for the regulation of growth in the root (Chen, 2006). Extensive work has shown that Al causes inhibition in root growth, root elongation, morphological disorganization in the root apex, root bending and an alteration in root anatomy (Yang *et al.*, 2015). Root apices generally play a pivotal role in Al^{3+} perception and response (Horst *et al.*, 2010; Silva *et al.*, 2012). Al^{3+} accumulated in root cell walls exerts a toxic effect in three ways: (i) it may decrease apoplastic exchange of basic cations, especially Ca, which could reduce nutrient acquisition per unit root length,(Bose et al., 2011), (ii) Al^{3+} absorbed in the cell wall reduces cell expansion, thereby reducing root elongation (Tabuchi and Matsumoto, 2001), and (iii) a reduction in nutrient-uptake through decreased root proliferation through the soil (Horst *et al.*, 2010). Inhibition of root growth is one of the earliest and most apparent symptoms exhibited by plants suffering from Al stress, this symptom is observed within hours or even minutes of exposure to a very low concentration of Al (Dipierro *et al.*, 2005; Ma, 2007). However, prolonged exposure of plants to Al, exhibited a number of toxicity symptoms in both roots and shoots (Rengel *et al.*, 2015). Al binds strongly with pectins present in the cell wall of epidermal and cortical cells of roots causing the primary injury of peripheral root cells, where it interferes with cell division at the root apex and lateral roots, that increases the rigidity of the cell wall by binding with pectins and inhibits the DNA replication because of rigidity of double helix (Eekhout *et al.*, 2017; Zhang *et al.*, 2014).

Recently, Cosgrove,(2015) reported that expansins a wall loosening proteins, play a role in plant growth and responses to abiotic stress while extensions involved in root hair morphogenesis and elongation. Guo *et al.*, (2017) suggested the down regulation of expansins and extensions might play a role Al-induced inhibition of root growth and Al-sensitivity in *C. grandis*. However, it is demonstrated that an Al-inducible expansin gene, OsEXPA10 play an important role in root growth but its response to high Al-tolerance is less in rice.

A. Al-induced DNA Damage-

Al also induced inhibition of mitosis in the root apex has been implicated blockage of DNA synthesis (Horst *et al.*, 2010; Jaskowiak *et al.*, 2018; Silva *et al.*, 2012), aberration of chromosomal morphology and structure, occurrence of anaphase bridges and chromosome stickiness and also programmed cell death occurs in the root tips (Pan *et al.*, 2001). Al also inhibits the number and length of lateral roots. Eekhout *et al.*, (2017) identified *Arabidopsis* mutant with high Al tolerance justifies the DNA as one of the main targets of Al toxicity. Al tends to bind with the negatively charged phosphodiester backbone of DNA and resulting in conformational changes in the DNA topology from the B-DNA to Z-DNA with subsequently increased DNA rigidity that leads to resistance in unwinding during DNA replication and susceptibility of DNA to endogenous mutagens. It has been also reported, Al toxicity possibly alters the regulation and expression of the nuclear proteins leading to inhibition of DNA synthesis, consequently DNA fragmentation and formation of micronuclei. Al-induced DNA damage reported in *Arabidopsis* (Rounds and Larsen, 2008), and Barley (Jaskowiak *et al.*, 2018) promoted the activation of cell cycle arrest resulting root growth inhibition.

B. Al-induced P and S-Deficiency-

Roots are the main organs for uptake of nutrients in the plants from the soil, so Al toxicity inevitably affects the ability of plants to promote the uptake of nutrients from the soil thereby Al directly disturbs the transport and metabolism of nutrients within the plants (Zhao *et al.*, 2014). Moreover, Al tends to bind with phosphorous in less available and insoluble forms in soil and plants root by triggering a P deficiency for plants growth (Bojórquez-Quintal *et al.*, 2017; Silva *et al.*, 2012). Evidences showed that P-deficiency the key factor for the Al-induced growth inhibition in plants (Quartin *et al.*, 2001) and P supply could mitigate Al toxicity in plants. Guo *et al.*, (2017a) isolated 16 upregulated and 6 downregulated, and 18 upregulated 3 down-regulated low P-responsive genes from *C.grandis* and *C.sinensis* under Al stress. The supplementation of acid phosphatases (APs) is an adaptive strategy of plants under P-deficiency. Purple APs (PAPs, a major group of nonspecific APs) play a necessary role in Pi cycling and scavenging in the P-deficient plant (Liu *et al.*, 2016). Similarly sulphur mediated (S-mediated) alleviation of Al toxicity reported in Barley (Dawood et al., 2012) and wheat. S metabolism is the key pathway for the biosynthesis of molecules important for plant growth and development, contributing tolerance to biotic and abiotic stress including Al (Jiang *et al.*, 2015; Yang *et al.*, 2007). Dawood *et al.*, (2012) and Guo *et al.*, (2017a) reported that increased uptake of Ca, Mg and P responsible for the S-induced alleviation of Al toxicity in Barley and *C.grandis* seedling. Guo *et al.*, (2017a) also suggested that genes regulated in S-transport and metabolism might contribute to Al tolerance by increasing cell S level and biosynthesis of S-containing molecules responsible for Al detoxification.

2.2. Impact of Al Toxicity on Photosynthesis and Chlorophyll Content

The most common response of Al toxicity in shoots are cellular and ultrastructural changes in leaves, reduced stomatal opening, decreased photosynthetic activity leading to chlorosis of leaves (Guo *et al.*, 2012; Rouphael *et al.*, 2015). Fresh and dry mass of roots and shoots, leaf number and root/shoot ratio were also reduced by Al treatment.

Al stress induced a reduction in the quantity of chlorophyll pigment and the ratio of chlorophyll 'a' and 'b' which was accompanied by the marked decline in photosynthetic rate (Yang et al., 2015). Pereira et al., (2010) demonstrated that enzyme (ALA-D) involved in chlorophyll regulation is sensitive to metals due to the sulfhydryl nature and catalyzes the two molecule of aminolevulinic acid (ALA) to porphobilinogen and this enzyme requires three Mg ion for binding. In addition to this Al reduces the uptake of nutrients and interferes with the absorption and translocation of several cations eg. Ca and Mg. This is in accordance with the decrease in chlorophyll content under Al toxicity is caused by a decrease of biosynthesis of chlorophyll, ALA-D activity, and Mg. It also suppressed photosystem I mediated electron transport and stimulated photosystem II catalyzed electron flow and O2 evolution (Guo et al., 2017a). Photosynthetic rate of maize seedlings grown in acid soil was reduced by high Al concentration (Zhao et al., 2017). However, the reduction of photosynthetic activity in citrus species was attributed to the accumulation of Al in the leaves (Guo et al., 2017b). In presence of excess Al with low Ca and Mg resulted in stomatal closure and thereby also decreased photosynthesis in beech seedlings (Ridolfi and Garrec, 2000). In addition, Al may reduce the amount of almost all organic nutrients of plants (Matsumoto, 2000) and may interfere with the absorption, transport, and use of several cations such as Ca and Mg (Pereira et al., 2010; Zhao et al., 2014). Moreover, workers also reported that Al toxicity specifically inhibited the photosynthetic apparatus in many plants (Dawood et al., 2012; Zhao et al., 2017).

2.3. Impact of Al toxicity on Respiration Rate
Al is reported to decreases root respiration by interfering with enzymes contributing the deposition of polysaccharides in the cell wall and altered the activity of hydrolytic enzymes present in the Golgi apparatus and decreases the synthesis and transport of cytokinin (Bojórquez-Quintal et al., 2017). The total respiratory rate decreased with increasing supply of Al in rice, these circumstances were accompanied by a reduction in soluble carbohydrates including reducing sugars which formed the substrate for respiration. Soluble sugars also increased in sorghum treated with an elevated level of Al. The rice plants growing in presence of excess Al accumulated sugars which serve as an adaptive mechanism in maintaining a favorable osmotic potential (Mishra and Dubey, 2008). In Al-tolerant rice majority of Al-responsive proteins related to glycolysis is upregulated (Arenhart et al., 2014), thus the increased glycolysis in C. sinensis roots responsible for the Al-tolerance by increasing root respiration and energy requirement (Guo et al., 2017b). It was earlier reported that energy and carbohydrate metabolism exhibit the higher adaptive response to Al in C. sinensis than C. grandis (Jiang et al., 2015). Phosphoenolpyruvate carboxykinase (PEPCK) a key regulatory enzyme in gluconeogenesis play important role in the catabolism of malate/citrate pathway during fruit ripening and the downregulation of PEPCK promote malate/citrate accumulation in Al-induced C. sinensis, hence conferring Al-tolerance of C. sinensis (Guo et al., 2017b).

2.4. Impact of Al Toxicity on Nitrogen, Nitrate and NR activity
Acidic soils are characterized by less nitrification and high concentration of soluble Al, on the other hand, neutral and calcareous soils exhibit high nitrification and lower concentration of soluble Al. It was suggested that Al reduced the nitrate uptake in plants due to the internal binding of Al to membrane channel proteins or other components of nitrate transport system. Roots of cucumber seedlings supplied with the higher concentration of Al exhibited a low rate of nitrate uptake (Jerzykiewicz, 2001). The reduction of nitrate, absorbed by roots is initially catalyzed by nitrate reductase (E.G. 1.6.6.1), located in the cytosol of the cell, which reduces nitrate to nitrite. The reduction in the activity of NR under Al stress may results from the Al-inhibition of nitrate uptake, as the decrease level of substrate nitrate (Zhao and Shen, 2018). However, in some reports, Al had a stimulatory effect on NR activity at low concentration such as in, rice (Sharma and Shanker Dubey, 2005), Quercus serrata (Tomioka et al., 2012), and tea (Hajiboland et al., 2013). Recently, (Zhao and Shen, 2018) reported that the activity of NR is concentration dependent it is inhibited by high Al concentration and stimulated by low Al concentration.

3. Biochemical Effect of Al Toxicity on Plants
3.1. Al-induced ROS Production
Al can elicit the expression of ROS in plants cell (Xu et al., 2011; Yin et al., 2010). The enhanced expression of ROS by Al toxicity is regulated by several mechanisms. In the apoplast, activated plasma membrane NADPH-oxidase is the key source of ROS in plants under Al stress condition. The Al transiently enhances the cytoplasmic Ca level which stimulates the activity of plasma membrane NADPH oxidase, resulting in the production of O_2^- and H_2O_2 in the apoplast. Al can regulate the Fenton reaction by coupling with other metals, including Cu, leading to the overproduction of OH radical (figure 4). Al toxicity is associated with swollen/dysfunctional mitochondria, fragmented vacuoles and pre-apoptotic nuclear structures, which may consequently lead to induce mitochondrial pathway to initiate programmed cell death. Al induces production of ROS is a complex process. The H_2O_2 produce in the apoplast due to the NADPH oxidase and Fenton reaction may be entered to the cytosl. In addition, Al can easily cross the plasma membrane and attributed to activate the Fenton reaction in the cytoplasm leading to increasing cytosolic ROS concentration. In addition, the acidification of cytosol by Al may also be induce increased production of ROS in the cytosol and mitochondria resulting in the disruption of redox metabolic activities by destabilizing NAD^+ and also inhibits the generation of excess ROS in the mitochondria. This disruption interferes with the regulation of $NAD(P)H/NAD(P)^+$ level in another cellular component including mitochondria. Several reports showed that Al-induced SOD activity in plants. On the other hand, PODs play an important function in oxidative cross-linking of cell wall extensibility, as a consequent increase in cell wall stiffening and decreases cell wall extensibility respond to Al-induced inhibition of root growth (Ma et al., 2012). Ma, (2004) and Maron et al., (2008) suggested that POD mediated oxidative cross-linking contributed to being a potential strategy of Al tolerance. Al-induced cell death of barley root cells associated with an increase in H_2O_2 production by POD characterized for the protection of root tip under Al toxicity through chelation of Al in the dead cells. Guo et al., (2017b) also reported that Al-induced up-regulation of the POD in C. sinensis responsible for Al tolerance of C. sinensis than C. grandis. These reactive oxygen species form hydroxyl radical that causes lipid peroxidation, protein denaturation, DNA

mutation (Guo et al., 2017b; Silva et al., 2012; Surapu et al., 2014) and inhibition of photosynthesis (Pereira et al., 2010). Al-induced oxidative stress in Nicotianatobaccum and Pisum sativum (Yamamoto et al., 2002), Triticum aestivum (Darkó et al., 2004), Vigna radiata, germinating barley seeds (Šimonovičová et al., 2004), Oryza sativa (Ma et al., 2007), Jatropha curcas (Chao et al., 2014) and citrus species by inducing production of ROS. Tobacco cells sensitive to Al had increased formation of ROS (Yamamoto et al., 2002), whereas roots of wheat plants sensitive to Al accumulated more superoxide and peroxide than Al-tolerant plants, leading to reduced oxidative damage (Darkó et al., 2004). Recently the study reported that Al-induced, Al- tolerant C. sinesis root and leaves accumulated more ROS scavenging related proteins than the root and leaves of Al-induced intolerant C. grandis (Jiang et al., 2015; Li et al., 2016). However antioxidant enzymes also contribute a defensive mechanism in transgenic plants such as upregulation antioxidant enzyme genes conferred a degree of Al tolerance in canola plants higher expressing wheat manganese SOD (Basu et al., 2001) and transgenic tobacco plants higher expressing Arabidopsis cytosolic DHA reductase (Yin et al., 2010).

A. Al-induced H_2O_2 Production
More recently, H_2O_2 has identified also as a signal molecule that activates expression of many genes in plants (Desikan et al., 2000) and initiating several protective resistance mechanisms against biotic and abiotic stresses (del Río, 2015). H_2O_2 acts both as an oxidant as well reductant and plays a wider role in resistance reactions, as it is required for cross-linking of important components of plant cell wall (Lignin and Suberin) as a part of structural defense reactions and it may also regulate gene expression associated with antioxidant defenses (Apel and Hirt, 2004). In pumpkin (Cucurbita pepo) roots and germinating barley seeds, accumulation of Al in the root tip was correlated with the elevated level of H_2O_2 (Dipierro et al., 2005) further corroborated the involvement of H_2O_2 in defense reactions. Elevated levels of H_2O_2 could accelerate the process like Haber-Weiss reaction, resulting in the formation of hydroxyl radicals that caused increase lipid peroxidation in plants (Neill, 2002) as in Oryza sativa (Ma et al., 2007).

Al stress upregulated the expression of several genes to mitigate the ROS-induce damages that play an important role in tolerance of the plant against oxidative damages (ROS stress) (Zhou et al., 2017). Molecular approaches revealed that Al triggers the expression of various genes to detoxify ROS, and stimulates the recovery from ROS-induce damage. Some of these ROS-mediated genes play an important role in Al tolerance mechanism. Al-tolerance in A. thaliana and various crops are regulated by the ectopic expression of several ROS mediated genes suggesting that genes encoding ROS scavenging proteins may be helpful in breeding transgenic crops that are tolerant to Al toxicity. For instance, transgenic A. thaliana plants overexpressing three glutathione S-transferase genes and two peroxidase genes

from tobacco, these are Al-inducible genes and were identified to be tolerant to Al stress conditions (Li et al., 2012). Similarly, in transgenic mustard plants, the ectopic expression of wheat WMnSOD1 confers Al tolerance. While the study suggested that the expression overexpression of alternative oxidase increases the Al resistance. However, the production of ROS reduces under stress conditions due to the changes in the regular electron transfer reaction of mitochondria to the alternative oxidative pathway. These findings suggested that Al induced ROS genes play an important role to protect the plants from Al-induced ROS-damages and induced ROS production can also regulate the transcription of several Al tolerance genes including H_2O_2 induces the transcription of AtALMT1 and AtMATE in A. thaliana (Liu et al., 2009; Yamamoto et al., 2003).

B. Al-induced Accumulation of Proline
The accumulation of proteinogenic amino acid proline in plants is a general response to various abiotic stresses. It is proposed that proline functions as an osmoprotectant, radical scavenger, stabilizer of macromolecules and a cell wall component. Proline accumulation is considered to activate antioxidant defense mechanism and also act as a source of carbon and nitrogen for rapid recovery from the stress (Dong et al., 2015; Surapu et al., 2014). Plants exposed to high concentration of Al, show increased proline content justify the stress conditions in mungbean (Ali et al., 2008) and cucumber (Fariduddin et al., 2013).

3.2. Al-induced Lipid Peroxidation
Oxidative stress induces the degradation of important molecules such as lipids, amino acids, proteins and carbohydrate resulting in the release of malondialdehyde (MDA) determined by the thiobarbituric acid reactive substance (TBARs) (Yamamoto, 2001). An increase in MDA content is a precise indicator of general oxidative damage in membrane lipids. Al enhanced Fe mediated peroxidation of lipids leading to the loss of plasma membrane integrity and eventually cell death (Cargnelutti et al., 2006). Roots exposed to an elevated level of Al also showed peroxidation of membrane lipids, loss of cell compartmentation and production of ROS (da Silva et al., 2006; Guo et al., 2017b; Shen et al., 2014). Moreover, Al enhanced the peroxidation of lipids in pea roots and cultured tobacco cells, cucumber, citrus species.

4. Mechanism of Tolerance to Al in Plants
Al-resistant plants have developed two characteristic mechanisms of tolerance to counteract with the deleterious effects of Al. One is mechanism of exclusion or resistance to Al is based on the function to exclude or reduce the entrance of Al from root symplasm, whereas other relies on the ability to tolerate symplastic Al which compartmentalizes Al in vacuoles or stabilizes them in order to reduce toxicity generated by Al (Bojórquez-Quintal et al., 2017; Zhu et al., 2013). Figure 1 shows the effect of Al toxicity in plants and mechanism of aluminum tolerance.
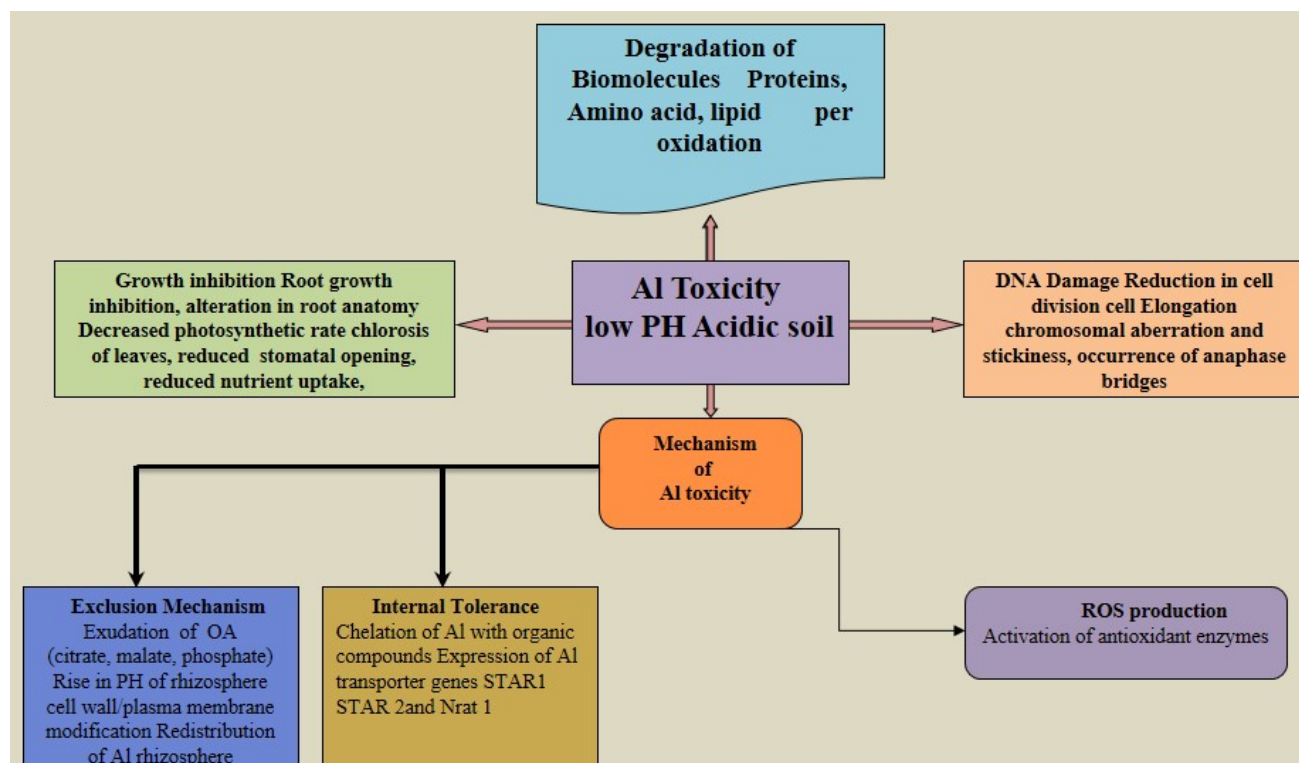
Figure 1: Effect of Al on plants and mechanism of Al tolerance. Exclusion mechanism and internal tolerance mechanism to stress by Al in plants and production of ROS through antioxidant enzymes

4.1. Exclusion Mechanism - It Involves Al-induced Excretion of Organic Acids.

Recent researches showed that the higher plants developed a defensive mechanism against toxicity generated by the high concentration of Al such as excretion of Al-induced organic acids (AOs eg. citrate , malate, and oxalate), phenolic compounds and phosphate (Pi) from the roots to the apoplast or rhizosphere is the most prevalent feature of Al-exclusion mechanisms in many crops plant (Yang *et al.*, 2011). However organic acid exudation from the roots is the best-characterized mechanism responsible for the Al tolerance in higher plants. Exclusion mechanism includes raising the pH in the rhizosphere, redistribution of Al, and modified cell wall is another mechanism was involved in mitigating the effect of Al toxicity through exudation of Al-chelating compounds (e.g organic acids) by restricting the entry of Al into the symplast.

A. pH Rise in the Rhizosphere

The content, solubility, activity, and the toxicity of Al of plants decrease through the exudation of Al in root apoplast with the increment in the pH of Rhizosphere (Bojórquez-Quintal *et al.*, 2017; Yang *et al.*, 2011). Several reports show that rise in apoplastic pH in *Arabidopsis* alr-mutant Al resistance and other plants species due to the H^+ and NH^+ influx and efflux of OA in the root apex (Bose *et al.*, 2010; Wang *et al.*, 2015). The increment in rhizosphere pH and resistance to Al toxicity are also regulated by H^+-ATPase of the plasma in wheat and *Cucurbita. Pepo* (Ahn, 2002; Yang *et al.*, 2011).

B. Modification in the Cell Wall and Plasma Membrane Properties

Cell wall serves as the first barrier of cellular Al uptake and characterized as a critical site for sensing Al toxicity and tolerance to Al. Horst *et al.*, (2010) reported that cell wall plays an important role in the manifestation and perception of Al toxicity. Several pieces of research reported that modification in the structure and /or function of cell wall responsible for the resistance to Al in plants. Al tends to change the structure of the cell wall, increases the rigidity and reduces the mechanical extensibility of cell expansion (Ma, 2004; Tabuchi and Matsumoto, 2001).

In plants polysaccharides, the content of the cell wall induced by Al can reduce the water and nutrients uptake, as well as cellular elasticity. The pectin content and its degree of methylation in the cell wall governing the differences in the resistance to Al. Higher pectin content stimulated to higher accumulation of Al in the cell wall (Yang *et al.*, 2011; Zhu *et al.*, 2013). Recently reported that instead of pectin the hemicellulose is the major component of the cell wall, that directly bind with Al in *Arabidopsis*. The function of enzyme xyloglucan endotransglycosylase that cut and rejoin xyloglucan chain is disrupted by Al consequently leading to cell wall loosening (Yang *et al.*, 2011; Zhang *et al.*, 2016) and downregulates the expression of xyloglucan enzyme (XTH14, XTH15, XTH31) such as, *Arabidopsis* with XTH31 exhibit less xyloglucan content and cell wall Al accumulation capacity and higher Al resistance (Zhu *et al.*, 2012).

To date, several pieces of evidence have indicated that the level of Al tolerance is species specific. Al tolerance is regulated by Al responsive gene expression (Tsutsui *et al.*, 2012). Recently several Al tolerance genes isolated which is responsible for the cell wall modification eg. STAR 1 and STAR 2 (sensitive to Al rhizotoxicity) encode an ATP binding protein and a transmembrane protein, respectively. The STAR 1 and STAR 2 complex transports UDP-glucose, which a substrate is serving as to modify cell wall and cover the Al binding sites. Nrat 1, a natural resistance-associated macrophage protein (Nramp), encodes an Al transporter, whereas FRDL 4, a multidrug and toxic compound extrusion (MATE) protein, regulate Al-induced citrate transporter used

in citrate secretion. Rice is one of the most Al resistance crops under field condition than wheat, sorghum or maize (Famoso *et al.*, 2010). ART1 are the transcription factor has been identified in rice and suggesting as an essential component of Al-responsive gene expression and regulates the expression of 31 downstream genes (Yamaji and Ma, 2009).

C. Exudation of Al-Chelating Compounds and Secretion of Mucilage

To cope with the deleterious effects of Al the mechanisms of exudation of OA and/or phenolic compounds is most widely described in plants (Kochian *et al.*, 2015). It is associated with the release of OA in the roots bound and limit the Al uptake in to the cytosol assisting internal detoxification by forming a non–toxic Al- complexes with organic compound in the cytosol, compartmentalization in the vacuole and generating ROS to protect the roots and permitting it to grow. In addition, Kochian *et al.*, (2015) have suggested the exudation of other organic compounds in the roots involved in chelation of Al. However, the possible mechanisms not clear. In tea plants, release of caffeine, a phenolic compound has been reported in response to Al detoxification. Other compounds released in roots to Al-resistance have also been reported such as phenolics compound (catechol, catechin, and quertin), flavonoids, succinate phosphate, ODP-glucose and polysaccharides in the form of mucilage (Bojórquez-Quintal *et al.*, 2017; Kochian *et al.*, 2015).

4.2. Internal Tolerance Mechanisms
A. Chelation of Al in the Cytosol

The internal Al tolerance mechanism involves the binding of Al entering the root cells as well as of Al in subcellular compartments (e.g vacuole) (Kochian *et al.*, 2015; Pereira *et al.*, 2010). The internal detoxification of Al is primarily based on the chelation of Al with OA and achieved via transport and subsequent sequestration/storage into the vacuole as Al-oxalate or Al-citrate complexes leading to the redistribution of Al within the cell. The over-accumulation of Al in plants facilitates the use of OA for the sequestration of Al in the cytosol of root cells and also to redistribute and translocate Al towards the shoots. It was demonstrated that the grafting cucumber onto pumpkin rootstock can restrict the Al root to shoot translocation throughout the sequestration of this toxic element into less sensitive parts of the plants and cell compartments (eg vacuole). The overexpression of FeIREG1 isolated from buckwheat responsible for Al-tolerance in transgenic *Arabidopsis* plants possibly through sequestration of Al into the vacuole (Yokosho *et al.*, 2016).

B. Al Transporters in the Plasma Membrane and Vacuolar Compartmentalisation

Biological membrane requires transport proteins for transportation. Al transportation through the plasma membrane and the vacuole tonoplast in plants has not been

extensively studied.however it has been proposed that the ABC transporters, transporters of binding to ATP, AtABCI16/AtALS3, AtABCI17/AtALS1 and OsALS1, as well as the Nrat (Nramp family) contribute to the detoxification of Al. Huang *et al.*, (2012) demonstrated that a tonoplast-localized Al transporter isolated in rice encoding the gene OsALS1 involved in sequestration of Al into vacuole and thus contributing to Al tolerance in rice. Rice is the most Al resistance cereal crop shows multiple strategies to cope with Al toxicity. However, reported that Al uptake in rice is mediated by OsNarat1 which remove Al from apoplast and sequester it into vacuole of roots by regulating with a vacuolar ABC- transporter OsAlS1. In addition, the expression of several genes regulating aquaporins, ABC transporters and major facilitator superfamily proteins (MFSs) was identified in *C. grandis* and *C. sinensis*, suggesting the possible role in Al sequestration (Guo *et al.*, 2017b). Beside this the RNA-seq (RNA-sequencing) technique is used to investigate the genes involved in Al toxicity in higher plants such as rice (Arenhart *et al.*, 2014), *Hydrangea acrophylla* (Chen *et al.*, 2015), Buckwheat (Yokosho *et al.*, 2014), and citrus species (Guo *et al.*, 2017b).

Molecular approaches revealed that in plants the expression of different genes regulated in response of Al resistance which codify the membrane transporter proteins and facilitate the release of OA anions. These are the characteristic member of the ALMT (Al-activated malate transporters) and MATE (multidrug and toxic compound extrusion) families. Guo *et al.*, (2017b) demonstrated that several transport-related genes were isolated from *C. grandis* and *C. sinensis* roots in response to Al resistance, for instance, the genes ALMT and MATE encodes for the malate and citrate transporter respectively and ferric reductase defective 3b (FRD 3B), ATPases, ion transporter, cyclic nucleotide-gated ion channels (CNGCs), ammonium transporter and amino acid transporters. Many ALMT 1 type genes have been reported in different plants, AtALMT 1), BnALMT 1 and Bn ALMT 2, HvALMT 1, ZmALMT 1. Table 3 shows Al-tolerance regulatory genes in various plants and their function.

5. Calcium in Plants

Ca is an essential plant nutrient present in the soil in relatively high concentrations. Ca is supposed to form the first line of defense in plants. It is required by the plants in its ionic (Ca^{2+}) form for a variety of structural roles, acts as a cytoplasmic secondary messenger, linking a range of external stimuli to their physiological responses, and in the vacuole plays a pivotal to counter cations for inorganic and organic anions (Bootman, 2012). In addition, Ca is also played an essential role in stabilizing the chlorophyll structure, facilitating the electron transport in photosynthesis and improving the efficiency of photosynthesis (Hochmal *et al.*, 2015) (Figure 2).
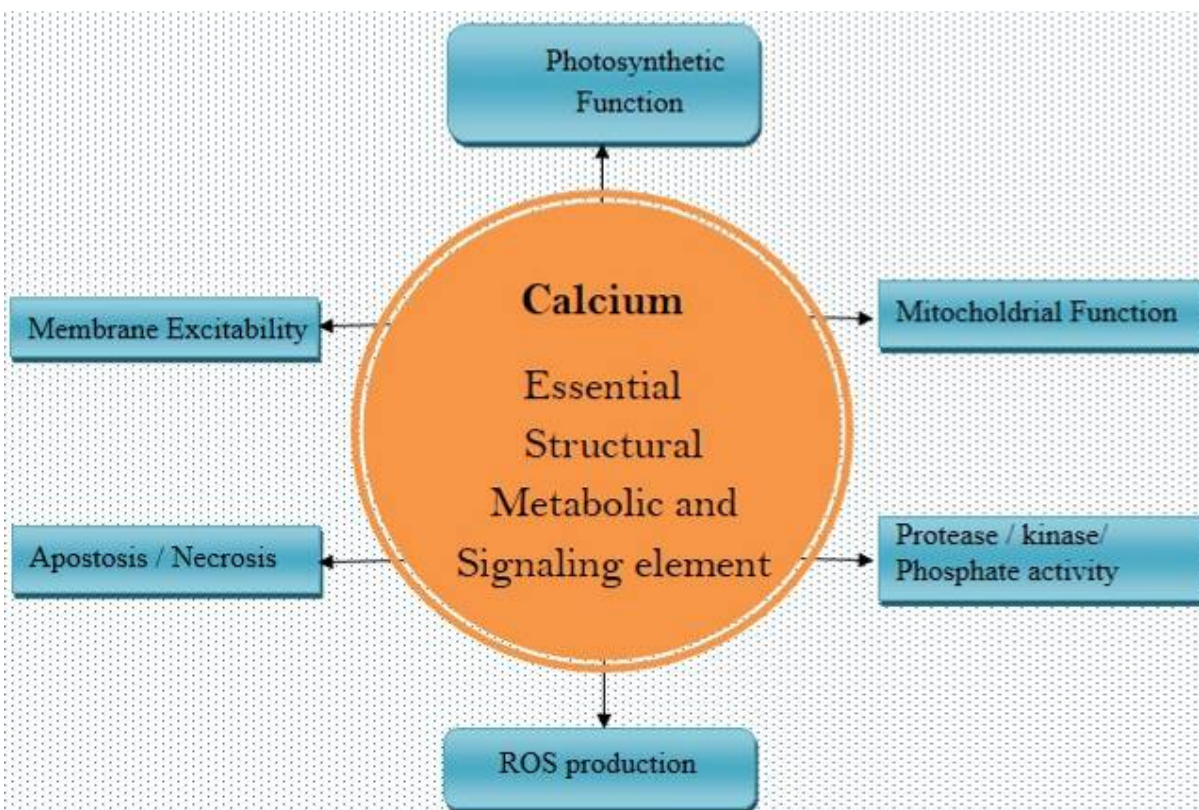
Fig.2 Protective functions of Ca in plants Ca are structural, metabolic and signaling element. Ca provides tolerance against Al by reducing Al uptake and producing ROS through stimulating antioxidant system and stabilizing cell membrane, maintaining photosynthetic rate and mitochondrial function, enhancing protease, kinase, and phosphate activity.

5.1. Cytoplasmic Ca^{2+}-Homeostasis

An elevated level of cytosolic Ca^{2+} is required by the expanding root hair cells and cells of elongation zone to maintain their proper growth. The cytosolic free Ca^{2+}concentration have been found to increase in response to abiotic stresses including salinity, cold, drought and heavy metals (Sanders *et al.*, 2002; Steinhorst and Kudla, 2014; Tuteja and Mahajan, 2007). The plant membranes are permeable to Ca^{2+} ions and it makes entry into the plant cells through Ca permeable cation channels located in the plasma membrane (Demidchik and Shabala, 2018; White, 2000). However, the rapid influx of Ca through cation channels in the plasma membrane, tonoplast and/or endoplasmic reticulum generates cyt [Ca^{2+}] perturbations that initiate cellular responses to a diverse range of developmental cues and environmental challeng (Demidchik and Shabala, 2018; Sanders *et al.*, 2002; White, 2000). Moreover, Ca^{2+} permeable channels in the plasma membrane play a pivotal role in cell signaling and in the regulation of a variety of growth, developmental, cell division, cytoplasmic streaming and photosynthetic processes (Hochmal *et al.*, 2015; Huang *et al.*, 2017).

5.2. Ca-Al Interactions in Plants

Al toxicity has frequently been linked to Ca^{2+} either because of induced perturbations in cellular metabolism of Ca or because of amelioration of Al toxicity by Ca^{2+} (Hossain *et al.*, 2014; Rengel and Zhang, 2003). Some of biochemical and physiological processes affected by Al-Ca interactions are: (i) disruption of cytoplasmic Ca^{2+} and pH homeostasis (ii) decreased the activity of H^{+}-ATPase in the plasma membrane coupled to depolarization of the plasma membrane, (iii) accumulation of callose and (iv) alteration of the cytoskeleton dynamics.

5.3. Role of Calcium Ion in the Implementation of Stress Protective Effect in Plants

The process of signal perception and transduction of Ca equipped with changes in the level of its intracellular free concentration that is used to coordinate a physiological response (Bender and Snedden, 2013). Ca accumulated in response to a distinct stimulus like biotic and abiotic and mediates a tight regulation of response reactions to various developmental processes or environmental challenges (Steinhorst and Kudla, 2014; White, 2003). In plants the potentially adverse conditions and continuous exposition of changing induce a distinct pattern of Ca levels in the cytoplasm in combination with highly regulated released and uptake from and intercellular stores and apoplast and therefore these modified encode information about particular stimuli referred as Ca signatures (Hochmal *et al.*, 2015; Steinhorst and Kudla, 2014). The Ca signaling mode comprises of variety of modules responsible, i) The generation of Ca signatures e.g increased level of Ca which is stimulus specific and may vary in terms of amplitude, frequency and duration, and shape, in response of stimulus (Dodd *et al.*, 2010; McCormack *et al.*, 2005). ii) Recognition of the signatures by Ca sensors, iii) transduction of Ca signatures information to targets that decoded signal specific responses (Edel and Kudla, 2015; Hochmal *et al.*, 2015; La Verde *et al.*, 2018). Ca sensors controlled the activity of downstream effector that mediate the changes in metabolisms, proteins, and gene expression (Reddy and Reddy, 2004). Generally, the Ca sensors possess a highly conserved helix-loop-helix motif referred to as EF-hand, and consist of 29 amino acids and a Ca ion that coordinates with 12 residues form a loop structure. In plants three types of Ca sensors identified e.g i) calmodulin (CAM) and CAM-like (CML), ii) calcineurin-B-like (CBL), iii) Ca-dependent protein

kinase(CDPKs) that sense, decode and covey the changes in [cyt] Ca in response to stress (Boudsocq and Sheen, 2013; Kudla *et al.*, 2010; Xu and Huang, 2017).

5.4. Regulation of the Ca2+-ROS hub (self- amplifying loop) in Al detoxification

It has long been known that the expression of environmental and particularly ion stresses in plants depends on the concentration of Ca^{2+} and Ca^{2+} ions alleviated toxic effects of various heavy metals by acting as an antagonist to those ions (Ma *et al.*, 2016). It has been reported that in the presence of toxicant supplementation with higher levels of Ca^{2+} alleviated growth inhibition. Furthermore, it was found that Ca increased metal resistance and also reduced the toxic effects of heavy metal in crop plants (Virdi *et al.*, 2015). It has been established that cytosolic free Ca^{2+} ions played a pivotal role by acting as the secondary messenger, in transduction of various hormonal and environmental signals (Pandey *et al.*, 2000; Plieth, 2001; Sanders *et al.*, 2002). Ca^{2+} also plays a role similar to plant hormone in the regulation of various cell functions in the plants. Moreover, it increases metal resistance by producing ROS (reactive oxygen species) and there is a connection between ROS and Ca^{2+} signaling pathways that enable the cell to cell communication and thereby long-distance transmission of signals in plants (Pottosin and Zepeda-Jazo, 2018; Steinhorst and Kudla, 2014). Recent data suggested that ROS generation and Ca^{2+} influx are also involved in diverse physiological responses including hormonal signal transduction, responses to stress, osmoregulation, programmed cell death, mineral uptake and long-distance signaling (Demidchik and Shabala, 2018; Demidchik et al., 2018). H_2O_2, one of the ROS, a signaling molecule an important factor of the signal transduction pathway that enables the plants to respond to varying environmental stimuli (Del Río, 2015). Pei *et al.*, (2000)

demonstrated that H_2O_2 involved to activate the Ca^{2+}-channels, thereby, inducing an increase cyt[Ca^{2+}] level that regulates the mechanism of Ca and CaM that may enable the plant to maintain the H_2O_2 homeostasis thus offering protection against stress-induced damage and play a possible role in metal detoxification (Virdi *et al.*, 2015). Recently it is reported that Ca activates the ROS production in plants through efflux of Ca^{2+} that activates the RBOH (respiratory burst oxidase homologs) via Ca^{2+} dependent phosphorylation by CDPKor CBL-CIPK signaling in response to different abiotic stresses. Other Ca^{2+}-permeable channels –NADP-oxidase (RBOHS) and their potential functions are summarized in table 4. On the other hand, the increase in level of cyt[Ca^{2+}] induces an increase in the NADPH oxidase-mediated production of ROS, vice-versa, the extracellular ROS enable to activate Ca^{2+} efflux through Ca^{2+}-permeable ion channels and this support the self amplifying Ca^{2+}-ROS mechanism (Demidchik and Shabala, 2018; Pottosin and Zepeda-Jazo, 2018). Similarly, the Ca^{2+}-dependent protein kinase activates by phosphorylation resulting MAPKs (mitogen-activated protein kinase) signaling cascade which induce cellular responses through the improved antioxidant system. MAPKs mediated ROS generation through enhanced activation of antioxidant enzyme was reported in *Arabidopsis* and rice (Liu *et al.*, 2010). Therefore, it may be concluded that Ca^{2+} ions, in addition to its role in cell signaling, also play a role in overcoming metal stresses in plants.

6. Brassinosteroids (BRs)

Plant hormones have a prominent role in monitoring various developmental, physiological and signaling cascades during abiotic stress (Choudhary *et al.*, 2012; Zhang *et al.*, 2016). A descriptive figure for the above mentioned is given in Figure3
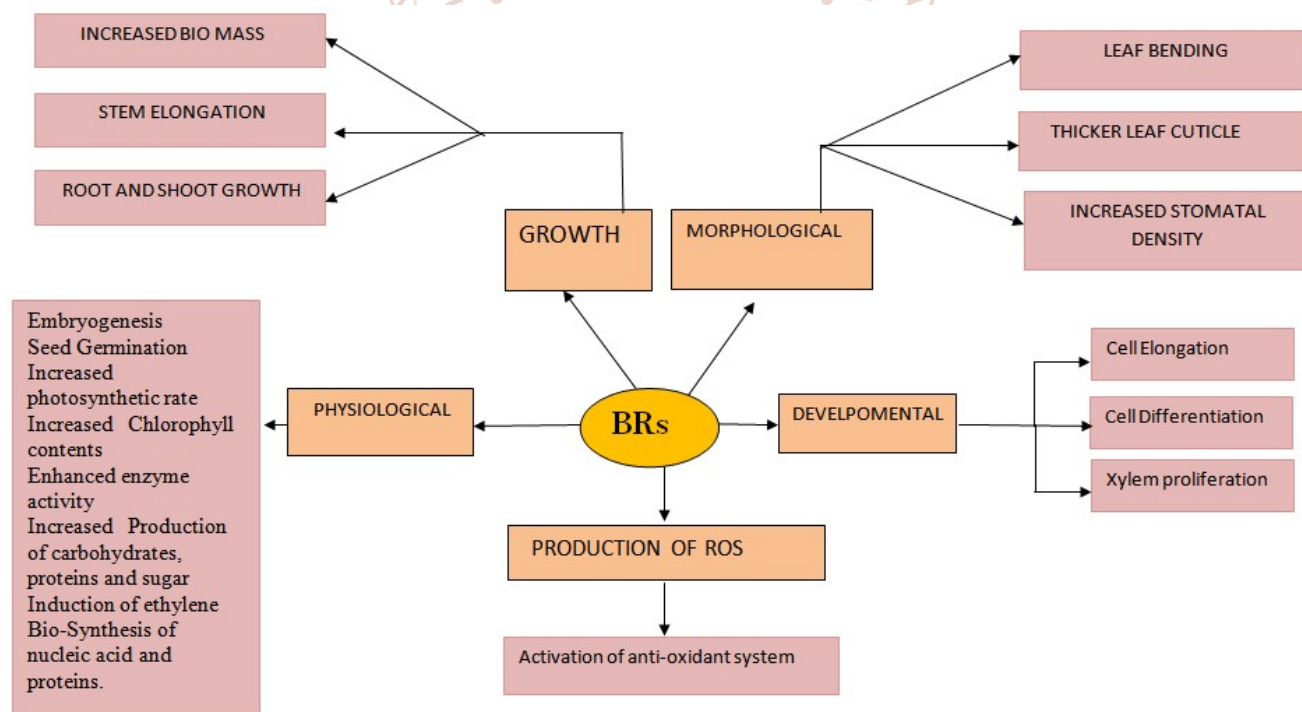

Figure 3: Role of Brassinosteroids in plants under Al stress. Brassinosteroids can regulate multiple functions in growth, development, physiology, and morphological aspects of plants. Brassinosteroids provides tolerance against Al by increasing ROS production through improved antioxidant system.

Brassinosteroids (BRs) represent a new class steroidal growth promoting plant hormone with the structure similar to animal steroidal hormones ecdysteroids and have a wide occurrence in the plant kingdom (Bajguz and Tretyn, 2003; Tang *et al.*, 2016). BRs are found to be involved in a wide array of biological activities in plants (Bajguz, 2010). They are involved in diverse physiological processes including seed germination, stem elongation, pollen tube growth, leaf bending and epinasty, induction of ethylene biosynthesis, proton pump activation, nucleic acid and protein synthesis, xylem differentiation, regulation of gene expression and senescence (Choudhary *et al.*, 2012; Zhang *et al.*, 2013). At present, more than 70 analogs of BRs are reported in plants. Among these analogs brassinolide, 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) are known to have an economic impact on plant metabolism, growth, and productivity and show more stability under field conditions (Khripach, 2000). Distribution of BRs is not uniform throughout the plant body though young growing tissues have collectively a large share than the mature tissue. However, BRs are present in all parts of plants including leaves, roots and also in cotyledons of seeds where they integrate various aspects of growth and development (Tang *et al.*, 2016).

6.1. BRs response to plants under stress conditions
A. BRs Response to Growth and Development in Plants
BRs has been reported to play stimulatory effect in growth, pigment contents , enzymatic activities and non- enzymatic antioxidant contents such as glutathione and ascorbic acid (Shahzad *et al.*, 2018; Vardhini and Anjum, 2015). The stimulatory role of BRs is possibly due to the progression of cell cycle and enhancement in cell division and elongation via upregulation of Xyloglucan endotransglycosylase (Zhiponova *et al.*, 2013).

B. BRs Restored Pigment Content and Photosynthetic Efficiency
The supplementation of BRs is also responsible for elevation in chlorophyll content associated with an enhancement in photosynthetic activity, by regulating the activities of the photosynthetic electron transport chain carrier and concentration of different protein-pigment–complexes. Exogenous supplementation of EBL to plants is reported to stimulate pigment content. Recently, Jan *et al.*, (2018) reported that exogenous supplementation of 24-EBL enhanced photosynthetic pigments and carotenoids contents due to its stimulatory effect on the activities of Ribulose 1, 5-biphosphate carboxylase, oxygenase and other enzymes associated with Calvin cycle and enhancing photosynthesis. Moreover, BRs are also acting as a key regulator in stomatal development (Acharya and Assmann, 2009). Reports of stomatal closure in the response of EBL were recorded in *Solanum lycopersicum* (Xia *et al.*, 2014), and also co-treatment of EBL+SA in *Brassica juncea* (Kohli *et al.*, 2018).

C. BRs Regulates Ion Homeostasis and Enhanced Mineral Uptake
Recently, the study of Ahmad *et al.*, (2016) and Waisi *et al.*, (2017) reported that application of EBL stimulates the absorption of essential inorganic ions and inhibit the uptake of toxic ions and regulate the ions homeostasis, especially K/Na, Ca and Mg in the upper leaves, Ca/Na and Mg/Na in the roots and K/Na in the petioles. Similarly, application of EBL improves nitrogen metabolism by maintaining ion homeostasis associated with the excessive translocation of Ca and Mg in the roots and shoots of cucumberseedlings (Yuan *et al.*, 2012). EBL also play an important role in Fe uptake by regulating Fe (lll) reduction to Fe (ll) and subsequently increasing Fe content in cucumber seedlings (Wang *et al.*, 2012). In addition, H^+-ATPase and Ca^{2+}-ATPase activity are also reported to enhance in response to EBL in the leaves of iron deficient pea plant (Song *et al.*, 2016). Jan *et al.*, (2018) also reported that co-application of EBL and Si enhanced the uptake of macronutrients and micronutrients in the root and shoot of *Pisum sativum* under Cd toxicity. Application of 24-EBL stimulates the uptake of Ca, Mg, Fe, And Zn content in salt stressed strawberry plant (Karlidag *et al.*, 2011), while folier application of EBL increased the K/Na and Ca/Na ratios in the salt-stressed wheat plant (Dong *et al.*, 2017). Thus, the growth regulatory response of BRs might be due to its role in ion homeostasis for biochemical or physiological processes (Karlidag *et al.*, 2011).

D. BRs Interaction with Other Phytohormones
To date, several pieces of evidence reported that BRs interact with other phytohormones such as ABA, GA, auxin, cytokinin, jasmonic acid (JA), Salicylic acid, and ethylene to regulate various physiological processes in an integrated manner (Choudhary *et al.*, 2012).

Previous studies reported that BRs and auxin interact synergistically to stimulate plant growth and transcriptional regulations (Saini et al., 2015). BRs can stimulate auxin-induced growth responses including root growth, hypocotyls elongation, laminar inclination, and shoot gravitropism (Bao, 2004).

It is also identified synergistic relationship observed between BRs and GA might be associated with the fact that they regulate the expression of MERI5, a XET thought to be involved in loosening of the cell wall. Both BRs and GA are associated in the regulation of plant photo morphogenesis, developmental processes and response to environmental signals (Zhu *et al.*, 2012).

The interaction between BRs and SA has been identified to be mainly involved in the regulation of plant responses to various environmental stresses. Recently studies show that co-application of BRs and SA can enhance plant salt tolerance in *Brassica juncea*. BRs and JA are reported antagonist in controlling plant growth reported BRs shows negative role in JA signaling pathway to suppress defense against root knot nematode in rice plant (Vidhyasekaran, 2015).

Recently the introduction of BRs and Cytokinin has been observed that BRs are involved in the Regulation of cytokinin levels in the wheat plant (Straltsova *et al.*, 2015; Yuldashev *et al.*, 2012).

BRs also reported to involved with ethylene and auxin to control shoot gravitropism in *Arabidopsis* (Singh *et al.*, 2014). However, the involvement of BRs and ethylene in the regulation of ethylene-induced hypnotic growth was observed in *Arabidopsis* and Cd tolerance in tomato (Sanjaya *et al.*, 2008).

6.2. BRs Regulate Antioxidative system
A common consequence of most abiotic and biotic stress is an increased production of reactive oxygen species (ROS). Exogenous applications of BRs modified the activities of

antioxidant enzymes and increase the content of ascorbic acid, glutathione, carotenoids, abscisic acid under various biotic and abiotic stresses (Bajguz, 2010). Another possible reason for the regulation of antioxidant enzyme activity might be BRs signaling receptor kinase (BSK 1) which trigger the level of SA and improve the effect of an antioxidant system (CAT, POD, SOD) by the formation of ROS (Dong *et al.*, 2015). BRs treatment enhanced the activity of catalase which scavenges H_2O_2 by converting it into H_2O and O_2. However, in contradiction to the above BRs reduced the activity of peroxidase and accumulation of H_2O_2 in plants (Apel and Hirt, 2004). Several reports of EBL mitigated oxidative stress have been proposed in plants such as in *Raphanus sativus* (Choudhary *et al.*, 2012), *Cicer arietum* (Ahmad *et al.*, 2016), *Brassica juncea* (Kohli *et al.*, 2018). BRsinduced enzymatic activities are associated with the regulation of genes involved in the gene expression of SOD, APX and CAT activities (Kaur Kohli *et al.,* 2018).

A. BRs Induced Reduction in Membrane Peroxidation
BRs are also reported to protect the membrane structure/stability under stress condition (Fariduddin *et al.*, 2013; Hayat *et al.*, 2010). However, BRs can regulate the activity of proteins and other membrane associated enzymes, either by changing protein conformation or protein function by direct interactions of proteins and sterols (Lindsey *et al.*, 2003; Rajewska *et al.*, 2016).
Brassica juncea treated with EBL under salinity and Ni stress exhibited decreased peroxidation of membrane lipids (Ali *et al.*, 2008). The similar results obtained by the study of Fariduddin *et al.*, (2013) that the ROS accumulation and lipid peroxidation decreased significantly by the application of BRs through the improved antioxidant system. In addition, Dong *et al.*, (2017) reported the application of EBL in salt-stressed wheat plants to decrease the MDA content to protect from the membrane damage.

B. BRs Induced Secretion of Proline
Recently it is reported that BRs mitigate the inhibitory effect of different stresses on the plant growth due to the increased level of protein and proline. Proline level has been reported in, mustard and chickpea (Ali., 2007) under NaCl stress, mungbean under Al stress (Ali *et al.,* 2008) and *Lycopersicon esculentum* under Cd (Hayat *et al.*, 2010), *Brassica* under cu stress(Fariduddin *et al.*, 2009) and cucumber under salt/Cu (Fariduddin *et al.*, 2013), wheat under salt stress (Dong *et al.*, 2017) were observed by the application of BRs.

6.3. BRs Ameliorative Response to Abiotic Stresses
BRs are well reported to have an ameliorative effect on plants subjected to diverse environmental stresses such as drought stress (Hu *et al.,* 2013), cold stress (Hu *et al.,* 2010), heat stress (Zhang *et al.*, 2013), oxidative damage and heavy metal stress (Fariduddin *et al.,* 2015; Hayat *et al.,* 2010; Shahzad *et al.,* 2018; Soares *et al.,* 2016). Applications of BRs have been reported to accumulate metals thus inhibiting the toxicity generated by metals such as Cd, Cu, Pb, Al, and Zn in various plants eg. tomato, barley, radish (Hasan *et al.,* 2011; Hayat *et al.,* 2010; Ramakrishna and Rao, 2015). Table 5

shows the BRs mediated response in plants under abiotic conditions.

Recently several reports demonstrated that BRs could enhance salt tolerance in various plants such as rice (Sharma *et al.,* 2013), strawberry (Karlidag *et al.,* 2011), and *Arabidopsis thaliana* (Derevyanchuk *et al.,* 2015). It was reported that EBL enhance the activity of antioxidative enzymes and antioxidant content stimulates plant growth under drought stress (Li *et al.,* 2012). In addition, BRs also decrease the malondialdehyde content and electrical conductivity of leaves under drought stress and enhance biomass production and seed yield in drought stress soybean. Moreover, Singh and Shono, (2005) demonstrated that application of EBL on tomato plant subjected to heat stress found more tolerant to heat due to higher accumulation of heat shock proteins induced by EBL under temperature stress and also improved photosynthetic efficiency. However, EBL in tomato plant protects the RUBISCO and other enzymes involved in Calvin cycle under heat stress (Ogweno *et al.,* 2010). Another study indicates the use of EBL in cucumber seedlings subjected to chilling stress enhanced the activity of RUBISCO and expression of photosynthetic genes.

6.4. BRs Mode of Action and Regulation in Stress Tolerance
Considering, high variability in BR's physiological role the two main aspects of the mechanism of action has been proposed, i) the impact of BRs on the biosynthesis of various enzymes through the activation of gene expression. ii) The impact of BRs on membrane functions (Fariduddin *et al.,* 2014; Wang *et al.,* 2014).

Recently, genetic and biochemical studies led to the recognition of BRs signaling cascade that BRs can modulate the activity of proteins and enzymes associated with membrane function either by changing their activity and confirmation through the direct interaction of proteins and sterols (Lindsey *et al.,* 2003). It is reported that BRs are recognized by a protein complex that comparises various BR-specific genes and receptors and certain stress responsive genes. BRs mediated signal transduction perceived by BRs protein complex includes the leucine-rich repeat receptor –like kinase encodes by Brassinoseroids–insensitive 1 (BRI1) localized at plasma membrane and mediate peptide signals and thus contributing in stress tolarence (Wang *et al.,* 2014). In the presence of BRs, BRs directly bind to the BRI1 at the cell surface and activate a signal transduction cascade that leads to the activation of two key transcription factor BZR1 (Brassinozole resistant 1) and BZR2. Among the BRs, the most active EBL and HBL tend to bind with extracellular domain of BRI1 receptor may result in activation of NADPH oxidase to ROS production, which probably mediate a cascade of protein phosphorylation through MAPKs to regulate transcription factor to target specific gene expression associated with cellular protection under stress conditions as shown in figure 4. However, molecular mechanism of BRs regulation of stress tolarence remain poorly understood.
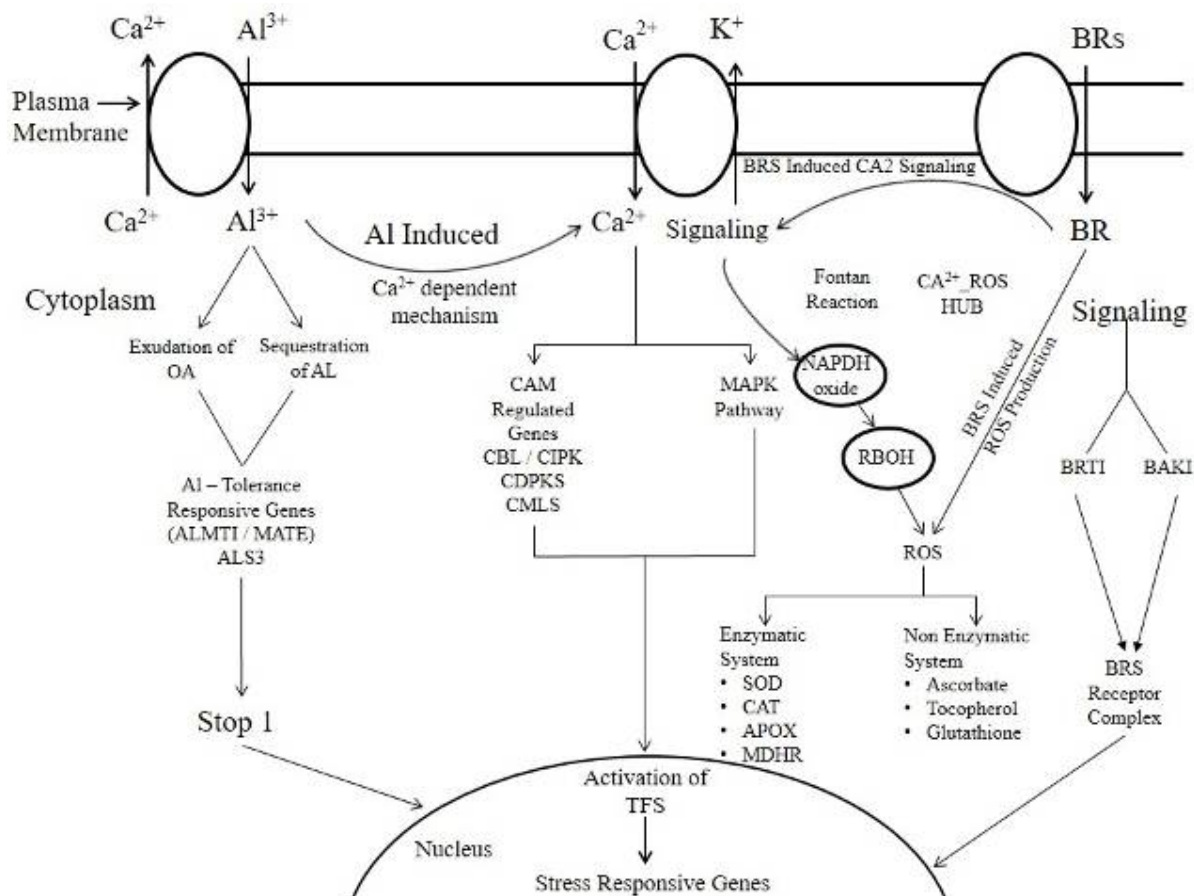
Figure 4: The hypothetical representation of Al stress signaling cascades and operation of ROS-Ca^{2+} self amplifying hub through the existing interaction among BRs and Ca^{2+} signaling in stimulation of reactive oxygen species (ROS) by NADPH oxidas, activated by cytosolic Ca under Al toxicity in plant.

6.5. BRs induced Calcium Dependent Regulation of ROS Generation (Plant Al Resistance)

The crosstalk between the BRs and Ca has been shown to be mainly involved in the regulation of plant responses to environmental stresses. More recently studies established the important role of the highly integrated module of BRs induced Ca^{2+}-ROS hub in connecting the BRs-Ca interaction and also mediating plant growth and responses to environmental signals (figure 4). It was shown that BRs capable of triggering a transient increase in the cyt [Ca^{2+}] which is equivalent to Ca^{2+} signals produced in response to biotic and abiotic stress-inducing factors. Therefore BRs play a critical role in the induction of Ca^{2+} signals during stress responses, elongation growth, developmental processes, gravitropic reactions and other Ca regulated processes. It was reported that BRs can also be regulated by plasma membrane system facilitate the influx of cyt [Ca^{2+}] in the leaves of *Arabidopsis thaliana*. Studies revealed that nitrogen oxide, reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) cascade are associated with the regulation of BRs responses followed by the development of resistance to cold and paraquat. Intriguinly, some studies show that BRs biosynthesis is regulated by Ca/calmodulin.

NADPH-oxidase (EC 1.6.3.1) is one of the key enzymes that generate ROS in plant-animal tissues. The activity of NADPH-oxidase is known to be activated by Calcium-dependent mechanisms. However, the possible mechanism of BRs induced interaction between ROS and Ca^{2+} signaling in resistance of plants to stress-inducing factors is poorly understood. . It is demonstrated that activation of NADPH-oxidase by Ca^{2+} associated with the activation of Ca^{2+}-dependent protein kinase , which phosphorylates the catalytic subunits of NADPH-oxidase and thus enhanced its activity and suggested that phosphorylated catalytic subunit of enzyme NADPH-oxidases binding with EF-hands of Ca^{2+}-binding loop undergoes structural modifications and activated more efficiently following subsequent activation of NADPH-oxidase leads to increase in the production of ROS are attributed for induction of stress –protective role of BRs. Hence BRs-induced signaling activated by Ca^{2+} and ROS, regulate the stress-protective system particularly the antioxidant system and increase in the resistance of plant cells (Figure 4).

7. CONCLUSION AND PERSPECTIVE

Much interest has been shown recently in the importance of BRs that have the ability to improve yield quantity and quality of various crop species and also to protect plants against various biotic and abiotic in stress condition. Several strategies have been successfully applied to generate plants which are able to grow in metal contaminated soil and accumulate and tolerate metal stress. In addition, Ca has been reported to respond to several hormones or regulate their biosynthesis simultaneously and signaling to regulate diverse plant defense mechanism. This may contribute greatly to the exploration and application of BRs and Ca in plant stress resistance mechanisms. There is a piece of evidence showing BRs induced Ca signal transduction may result in activation of NADPH oxidase to ROS production, which probably initiates a cascade of phosphorylation through MAPKs/CDPKs to activate transcription factors to

specific genes participating in cellular protection. It may be suggested that the increase in the degree of resistance induced by BRs and Ca was reflected in the improvement of plant growth, photosynthesis, antioxidant enzyme activity, and related processes under Al toxicity. Considering the importance of BRs and Ca in the plant, further investigations of key regulators in signaling pathway and mechanism underlying the whole regulatory system needed. These processes hint at a complex web of BRs and Ca signaling effects on gene expression. Moreover there is future research needs to expand the knowledge about the possible role and mechanism of BRs and Ca mediated signal transduction in plants and how these mechanism are linked to generate a distinct contribution to cellular signalings involved to reduce the stresses generated by Al. It may be useful to elucidate trace nature of complex transcriptomic regulation of Al tolerance and its underlying molecular mechanisms can also help us design optimal strategies to increase crop yield and enhance performance under Al stress conditions. The challenges that we are expected to face in order to employ brassinosteroids at a large scale in the field is their high cost. However, the recent advancement in the chemical synthesis of BRs and their analog has led to economically feasible approaches that have brought large scale applications very near to the reach of farmers for improving yield. The application of BRs and Ca in phytoremediation is a desired subject of study.

Author Contributions

SA designed, planned, and prepared the draft manuscript, KD draw figure, and table and revised the manuscript, PW-R checked and resived the manuscript, AZR checked the manuscript.

Availability of data and materials

We will deposit the datastes in publicly available repositories.

Compliance with Ethical Standards
Consent of publication

Not applicable

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethical Statement

Our work complies to the ethical rules applicable for this general.

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List of abbreviation used:

NADPH	Nicotinamide adenine dinucleotide phosphate hydrogenase
RBOH	Respiratory burst-oxidase homologs
CBL	Calcinuerine-B-like
CDPK	Calcium-dependent protein kinase
CIPK	Calcinuerine-b-like-intracting protein kinase
CML	Calmoduline-like
MAPK	Mitogen-activated protein kinase
BRI 1	Brassinosteroids-insensitive 1
BAKI	BRI 1-associated receptor kinase
ALMTS	Aluminium-activated malate transporter
MATE	Multi-drug and toxic compound extrusion
ALS	Aluminium sensitive 1
STAR 1	Sensitive to rhizotoxicity 1
STAR 2	Sensitive to rhizotoxicity 2
XET/XTH	Xyloglucan endotransglucosylase/hydrolases
ART	Aluminium -resistance transcription factor
XTH	Xyloglucan endotransglucosylase/hydrolases
FRD	Ferric reductase defective

CNGCs	Cyclic nucleotide-gated ion channels
ABC	ATP-binding cassatte
MFSs	Major facilitator superfamily protein
FeREG1	Iron regulated /ferroportin 1
Nramp	Natural resistance-associated macrophage protein
CAM	Calmoduline
STOP 1	Sensitive to proton rhizotoxicity 1
MGT	Magnesium transporter
MDA	Malondialdehyde
PEPCK	Phosphoenol-pyruvate carboxy kinase
FRD	Ferric reductase defective
TBRS	Thio barbituric acid reactive substance
SERK 3	Somatic embryogenesis receptor kinase
GA	Gibberellins
ABA	Absicic acid
SOD	Superoxidase dismutase
POD	Peroxidase
CAT	Catalase
NR	Nitrate reductase
MDHR	Monodehydro ascorbate reductase
APOX	Ascorbate peroxidase

Table1: Beneficial Effects of Al at Low Concentration in Plants.

Plant species	Al concentration(AlCl$_3$)	Effect	References
Oriza sativa	1.5 mM/L	Enhanced antioxidant activity	(Guo et al., 2012)
Arabidopsis	50 µM	Increased Mg uptake	(Bose et al., 2013)
Jetropha curcas	0.5,1, 2, 3 mM in vitro embryo	Enhanced antioxidant system	(Chao et al., 2014)
Cunninghmia lanceolata	0.5, 1.2, or 4 mM	Affect the absorption of H ,Ca, Mg, ion flow in the root	(Ma et al., 2016)
Barley	1mM	Increased frequency of cells in G2/M phase	(Jaskowiak et al., 2018)
Wheat	100 µM	Al content decreased with increasing Ca content in root is able to alleviate Al injury	(Hossain et al., 2014)
C. xalapensis	0.5 and 1mM	Inceased root biomass and number of lateral roots	(Gonzala-Santana et al., 2012)
Glyscine max	0 and 10mM	Increased elongation and activity of the root	(Yu et al., 2011)

Table2: Effects of Al Toxicity in Plants

Plant species	Al concentration	Effect	References
Rice	50mM(Al)	Chromosome abberetion , programe, reduction of amount of nuclear DNA,cell death	(Mohanty et al., 2004)
Arabidopsis	0.25, 0.75 and1.50 mM	Delayed cell division	(Rounds and Larsen, 2008)
Cucumis sativus	Particle bombardment with TaALMT1 gene	Increased ELP, H202, and enhanced protein and lipid peroxidation	(Pereira et al., 2010)
Quercus serratum	1mM (Al)	Stimulatory effect on NRA activity	(Tomioka et al., 2012, 2007)
Rhy	1.11 and 1.18mM (Al)	Decreased photosynthetic rate	(Silva et al., 2012)
Sorghum	2.4 µM	cell damage and generation of reactive oxygen species specifically in the root distal-transition-zone (DTZ),	(Sivaguru et al., 2013)
Eucalyptus	4.4mM (Al)	Decreased photosynthetic rate	(Yang et al., 2015)
C. grandis C.sinensis	0 and 0.1 mM	Alteration in cholorophyll,decreased RWC,ROS, ELP, soluble protein, MDA, and S-containing compound	(Guo et al., 2017a)
Maize	iTRAQ and 2D-liquid chromatography (LC) of Al- tolerant and Al-resistant	Decreased photosynthetic rate	(Zhao et al., 2017)
Rhygrass	0 and 0.2 mM(AL)	Trigger lipid peroxidation, and inhibit enzyme activities	(Pontigo et al., 2017)

Table3. Al-Tolerance Regulatory Genes in various Plants and Their Functions

PLANTS	REGULATORY GENES	PRODUCT	FUNCTION	REFERENCES
Arabidopsis	AtSTOP1	STOP1-like proteins	Regulates expression Of Al tolerance genes	(Iuchi et al., 2007)
Tobacco	NtSTOP1	STOP1-like proteins	Regulates expression Of Al tolerance genes	(Ohyama et al., 2013
Rice	VuSTOP1	STOP1-like proteins	Regulates expression Of Al toleranc genes	(Fan et al., 2015)
Rice	ART1	STOP1-like proteins	Regulates expression Of Al tolerance genes	(Xia et al., 2010)
Arabidopsis,	AtALMT1	ALMT1 (O.A transporters)	Al activated malate transporter	(Hoekenga et al., 2006)
Holcus lanatus	HIALMT 1	ALMT1	Al activated malate transporter	(Chen et al.,2013)
Sorghum	SbMATE	MATE	Al activated citrate transporter	(Magalhaes et al., 2007)
Arabidopsis	AtMATE 1	MATE	Al activated citrate transporter	(Liu et al., 2009)
Rice	OsFRDL4	MATE	Al activated citrate transporter	(Yamaji and Ma, 2009)
Rice	VuMATE 1	MATE	Al activated citrate transporter	(Fan et al.,2015)
Arabidopsis	AtALS3	ALS3	UDP-glucose transporter	(Larsen et al.,2005)
Rice	OsSTAR2	ALS3	UDP-glucose transporter	(Yamaji and Ma, 2009)
Tobacco	NtALS3	ALS3	UDP-glucose transporter	(Ohyama et al.,2013)
Rice	OsMGT1	MGT	Magnesium transporter	(Chen et al.,2012)
Rice	OsCDT3	OsCDT3	Cys-rich peptide at PM (after binding to the PM)	(Xia et al., 2013)
Arabidopsis	AtGST	GST	Glutathione-S-transferase	(Ezaki et al.,2000)
Tobacco	NtPOX	POX	Peroxidase	(Ezaki et al.,2000)
Wheat	WMnSOD	MnSOD1	MnSOD	(Basu et al., 2001)

Table4. Table with Summary of functions of plasma membrane Ca^{2+}-permeable channels and NADPH oxidase whichh are associated with reactive oxygen species and Ca^{2+} signals mediated by ROS-Ca^{2+} hubs.

Plasma membrane Ca-permeable channel	NADPH oxidas	Function	Refrences
AtCNC3	ARBOHC	Root cell growth	(Foreman et al., 2003; Gobert et al., 2006)
AtCNGC18, AtGLR1.2 AtGLR3.7	AtBOHD AtRBOHF	Pollen tube growth	(Kaya et al., 2014; Michard et al., 2011)
AtCNGC5 AtCNGC6	AtRBOHD AtRBOHF	ABA signaling	(Mori, 2004)
DACC	NbRBOHB	Brasssinosteroides Singling	(Kolupaev et al., 2014; Straltsova et al., 2015)
AtCNGC14	AtRBOHD	Auxin signaling	(Peer et al., 2013; Shih et al., 2015)
AtCNGC2	AtRBOHD AtRBOHF	Jasmonate signaling	(Lu et al., 2016; Maruta et al., 2011)
AtGLR3.3	AtRBOHD	Salicylic acid	(Manzoor et al., 2013)

Table 5: BRs Mediated Response in Plants under Abiotic Stress Conditions

PLANTS	TREATMENTS	EFFECTS	REFRENCES
Mungbean	24-EBL/lowtemp	Recover the growth and upragulatedtheproteins involved in methionin assimilation, ATP synthesis, cell wall construction.	(Huang et al., 2006)
Arabidopsis thaliana/ brassica napus / egg plant	24-EBL/salt stress	Enhanced seeg germination	(Kagale et al., 2007)
Tomato	24-EBL/high temp stress	Protect the RUBISCO and other enzymes involved in calvin cycle and RUBP generation	(Ogweno et al., 2010)
Wheat	24-EBL/ salt stress	Enhanced the activation of RUBISCO and expression of photosynthetic genes	(Dong et al., 2017)
Cucumber	24-EBL/ chilling stress	Enhanced the activation of RUBISCO and expression of photosynthetic genes	(Xia et al., 2009)
Brassica juncea	28-HBL/Cu stress	Enhanced antioxidant enzyme activity and proline content	(Fariduddin et al., 2009)

Strawberry	24-EBL/ salt stress	Improve growth and biomass production ,increased K, Ca, Mg, Fe, and Zn content	(Karlidag et al., 2011)
Triticum astivum	28-HBL/ Ni stress	Improve seed germination ,length of root and shoot,	(Yusuf et al., 2011)
Arabidopsis	24-EBL+SA/temp stress	Enhanced expression of SA-regulatory gene NPR 1 for stress tolarence	(Divi et al., 2010)
Raphanus.sativas	28-EBL/ Ni stress	Enhanced oxidative enzyme activity	(Sharma et al., 2011)
Cucumber	24-EBL/ Fe deficiency	Act as antagonist in regulating Fe-deficiency induce FRO expression as well as Fe transporter from root to shoot	(Wang et al.,2012)
Tomato	BRs/ pantherene stress	Regulates secondary metabolism	(Ahammed et al., 2013)
Brassica juncea	BRS	Stimulates the production of antioxidant – tocopherol	(Biesaga-Kościelniak et al., 2014)
Raphanas sativas	24-EBL / Cd stress	Enhanced photosynthetic pigment concentration	(Ramakrishna and Rao, 2015)
Oryza sativus	BRs/ heavy metal stress	Enhanced the antioxidant enzymes	(P. Sharma et al., 2016)
7.1.1. Brassica juncea	24-EBL/metal stress	Improve seedling growth and chlorophyll content	(A. Sharma et al., 2016)
Wheat	24-EBL/Fe-deficiency	Increased K/Na ratio leading to growth	(Song et al., 2016)
Wheat	24-EBL /salt stress	Increase soluble protein and proline content , chlorophyll content enhanced antioxidant enzyme activity and H-ATPase activity	(Dong et al., 2017)
Brassica juncea	24-EBL+SA /Pb stress	Improve root/shoot length,and enhanced carotenoid, glutathione , ascorbic acid and tocopherol content, elevation in the expression CAT, POD, GR,DHAR, and GST genes	(Kohli et al., 2018)
Cucumis sativus	24-EBL/ salt/cu	Increase proline content	(Fariduddin et al., 2013)
Pisum sativum	24-EBL+Si / Cd stress	Enhanced antioxidant system, glyoxalase system, and macronutrient content	(Jan et al., 2018)