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Abiotic Stress Responses in Plants: Potential Targets on Studying Heavy Metal Stress tolerance in Bryophytes

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Abstract

Pollution of the biosphere with heavy metals has phenomenally increased since the commencement of industrial revolution. It poses several environment and health concerns. High regeneration and metal accumulation capacity the possibility of genetic transformation by homologous recombination extend the candidature of bryophytes as promising experimental models for heavy metal stress tolerance studies. Plants use several abiotic stress pathways which share common elements that are potential "nodes" for cross talks. Common elements/ molecules, which are likely to occur early in several stress response cascades in bryophytes seem to be the potential targets for heavy metal tolerance studies that can be worked out in future, at biochemical, protein and gene level.

1. Introduction

Heavy metals are defined as metals with a density greater than 5 g cm^{-3}. Most of the heavy metals are transition metals with an incompletely filled δ-orbital, present as cations under physiological conditions. Fe, Mo, Cu, Zn and Mn are biologically significant, while As, Hg, Ag, Sb, Cd and Pb have no known function as nutrients and seem to be more or less toxic to plants (Goldbold and Huttermann, 1985; Breckle, 1991; Nies, 1999). The two main reasons for heavy metal's presence in atmosphere are natural sources and anthropogenic activities. Natural sources include weathering of bedrocks, volcanoes and continental dusts. Anthropogenic activities like mining, combustion of fossil fuels, phosphate fertilizers, etc. lead to the emission and accumulation of heavy metals in ecosystem (Lanzty and Mackenzie, 1979; Galloway *et al.,* 1982; Angelone and Bini, 1992).

Heavy metals can have numerous harmful effects on organisms. The ions of some of these heavy metals readily form ligands with organic molecules and tear them. Heavy metals affect enzyme functions, cell membrane permeability and cell growth (Moriarty, 1999). Metal pollutants disturb the species, community, and ecosystem as a whole through food chain (Nriagu, 1996). Toxic effects of heavy metals are exerted at the extracellular as well as intracellular levels in bryophytes. Two different uptake routes have been identified: a) passive uptake, only driven by the concentration gradient across the membrane and b) active energy dependent uptake (Nies, 1999; Williams *et al.*, 2000).

Heavy metals can be divided into two groups of redox active and redox inactive metals. Autoxidation of redox active metals such as Fe^{2+} or Cu^{+} results in O_2^{-} formation and subsequently in H_2O_2 and OH^{-} production via Fenton-type reactions. Cellular injury by this mechanism is reported for due to heavy metals (Jones *et al.,* 1991; Lund *et al.,* 1991; Shi *et al.,* 1993; Imlay *et al.,* 1998). Another important mechanism of heavy metal toxicity is its ability to bind strongly to oxygen, nitrogen and sulphur atoms. Direct effect of cadmium on the sulphydryl homeostasis of cells and inhibition of enzymes has been reported for mammalian and animal cells (Chrestensen *et al.,* 2000). Heavy metals also harm by displacing the essential metal ions from enzymes. Divalent cations such as Co^{2+}, Ni^{2+} and Zn^{2+} were found to displace Mg^{2+} in ribulose-1, 5-bisphosphate-carboxylase/oxygenase and result in loss of activity (Van Assche and Clijsters, 1986; Rivetta *et al.,* 1997).

Plants have an inbuilt capacity to adjust to a variety of abiotic stress conditions. Abiotic stresses elicit complex cellular and molecular responses in plants that enable them to tolerate and survive adverse conditions. Plant's acclimatization to abiotic stresses is accompanied by a cascade of events which starts with stress perception and ends with the expression of a set of target genes eventually leading to a variety of morphological, biochemical and physiological changes. Plants use common pathways and components in a stress response relationship. This phenomenon is called cross-tolerance whereby a plant adjusts to a range of different stresses after being exposed to one specific stress. Separate abiotic stress signaling pathways are likely to interact in a similar manner i.e. "Cross talks" can occur between different sensors and signal transduction pathways. It could be any instance of two signaling pathways from different stressors that converge (Knight and Knight, 2001). Several abiotic stress pathways share common elements that are potential "nodes" for cross talks. Factors that act at early stages of a stress response cascade are critical for other cell functions and significantly affect the operation of other genes. There are common signals/elements, which are likely to occur "early in the stress response cascades" (Pastori and Foyer, 2002). During stress they show higher superoxide dismutase (SOD) activity or lower activities of the chloroplastic H_2O_2-processing enzymes peroxidase and ascorbate peroxidase. Significant decreases in the activity of catalase and depletion of the anti-oxidant ascorbic acid is also reported. The role of activated oxygen-processing enzymes involved in the removal of chloroplastic hydrogen peroxide may be less important than that of anti-oxidants in the determination of stress tolerance (Oliver *et al.*, 2005).

2. Cross talks between different pathways can be mediated at different levels

2.1. Elevation in cytosolic free calcium levels

Elevation in $[Ca^2]_{cyt}$ is thought to be the primary stimulus sensing event in response to different environmental stresses (Sanders *et al.*, 1999; Knight 2000). Plants exhibit markedly different levels of elevation in $[Ca^2]_{cyt}$ when already exposed to a previous stress indicating a cross talk between abiotic stress signal transduction pathways occurring at the level of calcium (Knight *et al.*, 1998; Knight, 2000). In *Arabidopsis*, an oxidative stress encounter nullified further response to drought stimulus (Knight *et al.,* 1998), while it increased the sensitivity of response to low temperature levels (Knight, 2000). Drought pretreatment increases the magnitude of subsequent drought induced $[Ca^2]_{cyt}$ transients and increases the level of drought inducible calcium regulated gene expression and stress tolerance (Knight *et al.,* 1998).

Calcium plays an important role in mediating plant environment interactions. While plants possess a unique repertoire of Calmodulin (CaM) related proteins of yet unknown function, the diverse processes in plants seem to be modulated by Calcium/CaM in response to signals from environment (Arazi *et al.,* 1999; Sunkar *et al.,* 2001). Owing to its ubiquitous nature in abiotic stress signaling, calcium signaling can be considered as a potential node at which cross talk can occur. Bryophytes have the ability to maintain the carbohydrate levels during the stress (Robinson *et al.*, 2000). Generally stress causes oxidative damage and it induces marks of oxidative stress. However, the activities of superoxide dismutase, peroxidase and catalase in the increase rapidly during stress period which indicate an inducible defense capability against ROS (Chen *et al.*, 2013). During the stress environments, plants transmit the signal to cellular machinery to activate adaptive responses. The ability of bryophytes to tolerate metal stress is determined by multiple biochemical pathways. Transmembrane transport proteins that mediate ion fluxes play a crucial role in ionic and osmotic homeostasis under stress environments. Defense proteins protect cells from denaturation and degradation, as well as from oxidative damage following exposure to salt stress in bryophytes. ABA and salt stress positively affect the expression of common genes that participate in protection plant cells from injure, and ABA may be responsible for the ability to tolerate stress (Wang *et al.*, 2008).

2.2. Calcium sensors

Calcium regulated effector proteins control various processes after calcium elevation. Calcium sensors might also serve as nodes at which cross talks can occur. $[Ca^2]_{cyt}$ elicited by specific stress could regulate phosphatase and kinase involved in transduction of other stimulus. A variety of calcium sensors have been recognized to play pivotal role in stress responsive signaling.

- CDPKs: Calcium dependent protein kinases. In rice, expression of OsCDPK7 at mRNA level is induced by cold and salt stress (Saijo *et al.*, 2000).
- Calmodulins: Calmodulin has been implicated in plant response to cold, mechanical stimulation, oxidative stress and heavy metal stress (Braam and Davis, 1990; Botella and Arteca, 1994).
- Calcium regulated phosphatases: In *Alfalfa* cells, cold induced inactivation of protein phospatase 2A (PP2A) is controlled by Ca^2 influx, but PP2A activity could also be modulated by other stress induced $[Ca^2]_{cyt}$ elevations (Monroy *et al.,* 1998). ABI1 and ABI2 proteins in *Arabidopsis* are potential nodes for cross talks between different signaling pathways involving ABA eg. drought and cold (Sheen, 1998; Sunkar *et al.,* 2001).

2.3. MAPK cascades

MAPK cascades are activated by numerous abiotic stresses (Ligterink and Hirt, 2001). Various abiotic stresses rapidly activate *Arabidopsis* MAP kinase ATMPK4 and ATMPK6 providing an evidence for cross talk between MAPK cascades leading separately to ATMPK6 and ATMPK4 (Ichimura *et al.,* 2000).

2.4. Transcription factors

Many genes that are induced by cold are also induced by drought or ABA (Shinozaki and Yamaguchi-Shinozaki, 2000), probably because many cold inducible genes encode proteins to protect the plant from the consequences of freezing stress, which includes dehydration. The gene *RD29A* has been utilized in several studies examining the convergence of these pathways (Yamaguchi-Shinozaki and Shinozaki, 1994). In *Arabidopsis*, two groups of transcription factors, DREB1and DREB2, bind to the cis acting elements (Stockinger *et al.,* 1997; Liu *et al.,* 1998; Shinozaki and Yamaguchi, 2000). The *DREB1* and *DREB2* genes encode structurally different proteins and are induced specifically by low temperature and by salt and drought, respectively. Overproduction of either DREB1 or DREB2 proteins in protoplast increased expression of an artificial RD29A-promoter GUS fusion gene (Liu *et al.,* 1998), indicating that the DREB promoter element is a point at which drought or salt and cold signal transduction pathways converge and that it can integrate information about these two stimuli.

2.5. Reactive oxygen intermediates

Oxidative stress is a common signaling event in all stress situations. Independent of the type of stress influence (natural or anthropogenic), the accumulation of reactive oxygen intermediates is an established fact. Reactive oxygen intermediates (ROIs) are partially reduced forms of atmospheric oxygen. They typically result from excitation of O_2 to form singlet oxygen [O_2^1] or from the transfer of one, two or three electrons to O_2 to form, respectively, superoxide radical [O_2], hydrogen peroxide [H_2O_2] or a hydroxyl radical [HO^-]. ROIs are capable of unrestricted oxidation of various cellular components and can lead to the oxidative destruction of cellular components (Asada, 1999; Dat *et al.,* 2000). It is well known that water deficit (Sell and Hendry 1992),

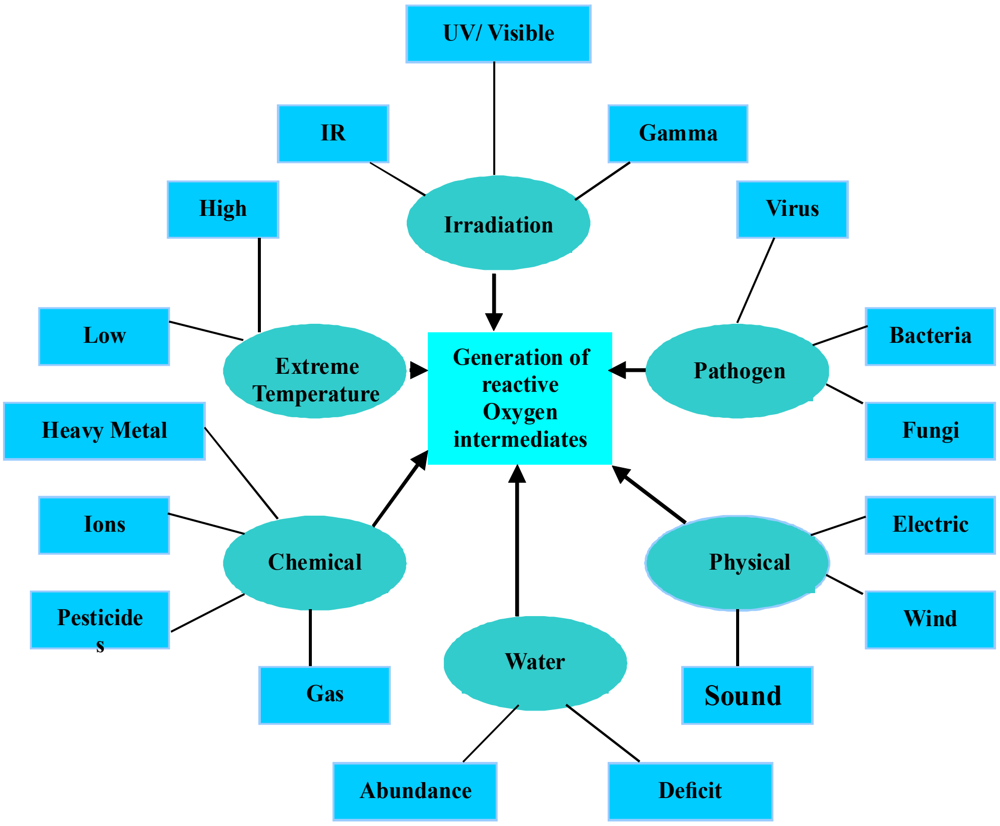

Fig. 1: Agents leading to generation of reactive oxygen intermediates (Modified from Alexieva *et al.,* 2003)

low temperatures (Badiani *et al*., 1993; Bridger *et al.,* 1994), radiation (Dunning *et al.,* 1994), heavy metals (Gille and Sigler, 1995), acid rains (Velikova *et al.,* 2000), high solar irradiation (Lichtenthaler, 1996) etc. cause an increased production of toxic oxygen species (O^2, H_2O_2, OH^-, O_2^1), which are highly detrimental to all biological systems (Fig. 1). Plants have developed complex antioxidant protective systems in order to cope with all these destructive effects.

Most important common response of plants to different abiotic stresses is accelerated production of reactive oxygen intermediates (ROI). They act as secondary messengers in stress response signal transduction pathways. Abiotic stresses enhance ROI production in chloroplast and mitochondria, while on the other hand by inducing ROI scavenging enzymes reduce ROI levels. ROI production takes place in mitochondria during reduction of respiratory electron transport chain (Moller, 2001) and scavenged by Ascorbate peroxidase (APX) or Superoxide Dismutase (SOD). In peroxisomes, ROIs are produced by glycolate oxidase and β-fatty acid oxidation. Catalase (CAT) is responsible for ROI detoxification during stress, when high level of ROIs is produced (Willekens, 1997). The cytosol with ascorbate glutathione cycle and peroxisomes with CAT act as buffer zones to control overall level of ROIs that reach different cellular compartments during stress and normal metabolism (Mittler, 2002; Mittler *et al.,* 2004). Balance between activities of CAT, SOD and APX in cell is essential for maintaining the steady state levels of ROIs which together with sequestering of metal ions prevent formation of highly toxic hydroxyl radicals via Fenton reaction (Asada and Takahashi, 1987; Willekens, 1997; Asada, 1999).

2.6. Redox mediated signaling

H_2O_2 acts as a signal-transducing molecule in optimal and stress conditions. This molecule is central to cross tolerance phenomenon and is a key component of stress survival network. Upon elicitation, H_2O_2 is produced in apoplasm by several processes including activation of NADPH oxidase, cell wall peroxidase or other related enzymes ie. SOD (Bolwell *et al.,* 1995). H_2O_2 has a strong regulatory influence on the fluxes through calcium channels and on calcium concentration in different cellular compartments. The life span of H_2O_2 in a cell depends upon two antioxidant buffers in a cell, namely, ascorbate and glutathione together with antioxidant enzymes that use these antioxidants (Pastori and Foyer, 2002). Ascorbate and glutathione remove H_2O_2 via the Halliwell-Asada pathway (Fig. 2) (Noctor and Foyer, 1998).

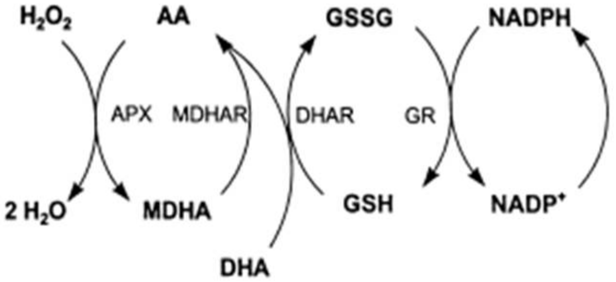

Fig. 2: Halliwell-Asada pathway or ascorbate glutathione cycle. APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase (May *et al.,* 1998)

Thus it can be concluded that signaling pathways leading to various responses to different stresses constitute a network that is somehow directly or indirectly interconnected at various levels. Various molecules play important roles in helping bryophytes combat a variety of environmental stresses. Bryophytes are simple and appropriate experimental models that enable the researcher to biochemically and genetically dissect out the responses of plants to heavy metal stress, which has become a major area of concern for the environmentalist and the mankind in general (Ingemar *et al.,* 2001; Yayintas *et al*, 2007).

3. Bryophytes as model systems for heavy metal stress tolerance studies

To be successfully employed as a biomoniter in a heavy metal contaminated zone, a plant ought to be fast growing besides having ability to accumulate large quantities of environmentally important metal contaminants. Bryophytes qualify as promising candidates for such studies. Widespread occurrence of these plants and their ability to accumulate metals has lead to their use in environmental monitoring programmes (Wittig, 1993; Conti and Cecchetti, 2001).

Bryophytes facilitate the assessment of overall environmental condition of a given zone (on the basis of stress analysis) alongwith the environmental concentration of a particular contaminant that is present (from tissue analysis). Furthermore, they integrate changes in concentration of metals through time, thereby providing biologically weighted average (Ruhling and Tyler, 1968; Puckett, 1988). Owing to their incredible regeneration capacity, any part of plant can regenerate a protonema in some species, under appropriate conditions. Genetic transformation studies in bryophytes have revealed that integration of foreign DNA sequences in the genome occurs preferentially at targeted location by homologous recombination

(Schaefer, 2002). Thus, the powerful genetic tools can be directly applied for various studies in bryophytes.

Changes in nitrate reductase activity and oxidative stress response in the moss *Polytricum commune* subjected to Chromium, Copper and Zinc phytotoxicity have been reported (Panda and Choudhary, 2005). Induction of oxidative stress and ultrastructural changes are reported in moss *Taxithelium nepalense* under Pb and As phytotoxicity (Choudhary and Panda, 2004). The mechanism of extracellular superoxide radical formation and the role of the oxidative burst in response to desiccation stress is reported in bryophytes and lichens (Minibayeva and Beckett, 2001). Gene structure and expression pattern analysis of three monodehydroascorbate reductase (*Mdhar*) genes in *Physcomitrella patens*, a key enzyme in ascorbate glutathione pathways, playing a major role in detoxification of ROI in plants have been published (Lunde *et al.,* 2006). Metabolism of Reactive oxygen intermediates in desiccation-stressed thalli of the liverwort *Dumortiera hirsuta* has been studied (Beckett *et al.,* 2004). Mayaba *et al.* (2002) reported an oxidative burst of hydrogen peroxide during rehydration following desiccation in the moss *Atrichum androgynum.*

4. Various possible targets for studying heavy metal stress tolerance in Bryophytes

4.1. Micro RNAs

Micro RNAs (miRNA) are one class of endogenous tiny (21-22 nucleotide) non-coding RNA that play important regulatory roles in plant development and response to external stimuli (Bartel and Bartel, 2003; Kidner and Martienssen, 2005). miRNA controls gene expression post transcriptionally by targeting cognate mRNAs for degradation (Llave *et al.,* 2002; Palatnik *et al.,* 2003) or by translational repression (Reinhart *et al.,* 2000; Aukerman and Sakai, 2003; Chen, 2004). To identify miRNAs, a library of small RNAs from protonema of *Physcomitrella patens* was constructed and several distinct sRNA expression patterns were observed (Arazi *et al.,* 2005). MiR156, Pp-miR319b, miR390, Pp_71 and Pp_89 were expressed at similar levels in the juvenile as well as adult phases of the gametophyte. Higher expression levels of Pp_42 and Pp_94 were observed in *P. patens* differentiating caulonema cells, while Pp_60 was detected almost entirely in non-differentiated protonema that is mainly composed of chloronema cells. It can be speculated that Pp_60 might function in an opposite way to Pp_94 and downregulates one of the factors that regulates chloronema differentiation (Arazi *et al.,* 2005). The transition from chloronema cells to caulonema is an indication of stress in bryophytes and thus miRNAs might serve as promising tools for stress tolerance studies in bryophytes (Schween *et al.,* 2003).

4.2. ALDH11A5 : a novel non-phosphorylating GAPDH cDNA

A cDNA ALDH11A5 (AY504666) was identified in *P. patens* with significant similarity to cytosolic NADP$^+$-dependent, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN; EC 1.2.1.9) (Wood *et al.*, 2004). GAPN is member of ALDH protein subfamily (Wood and Krayesky, 2002). ALDH11 (GAPN) orthologues operate in cytosol where enzymes generate NADPH for biosynthetic processes (Gao and Loescher, 2000). Increased GAPN activity allows glycolysis to continue under stress conditions that limit adenylate nucleotide concentration. GAPN glycolytic shunt continues to function in response to environmental stress (Plaxton, 1996). The expression level of ALDH11A5 was studied in response to osmotic stress, salt stress and ABA (Wood *et al.,* 2004). Further molecular and physiological experiments can evaluate the role of ALDH11A5 in plant development and adaptation to environmental stresses.

4.3. Sugar: 3-O-Methyl rhamnose

Primary cell wall (PCW) plays an indispensable role during stress tolerance in plant systems. One major wall related difference between bryophytes and higher plants is that bryophytes do not have lignified conducting tissues. The major sugar residues in PCW of bryophytes are galactose, mannose, xylose, arabinogalactan, rhamnose etc (Thomas, 1977). Although monosaccharides present in bryophytes are similar to those found in angiosperms, there are quantitative differences between PCW composition between bryophytes and angiosperms: bryophyte PCWs contain more galactose A, galactose C and mannose than those of angiosperms (Popper and Fry, 2003). Immunocytochemical studies of bryophyte cell walls indicate that they contain high concentrations of rhamnogalacturonan-I, homogalacturonan and arabinogalactan proteins (Ligrone *et al.,* 2002). 3-O-Methylrhamnose found in high concentrations particularly in bryophyte's primary cell walls may be associated with an acidic polysaccharide which helps in preventing dessication in bryophytes (Popper *et al.,* 2004).

4.4. IQD1: Calmodulin binding nuclear proteins

IQD1 are novel calmodulin binding nuclear proteins, which are known to stimulate glucosinate and plant defense in *Arabidopsis thaliana.* One contig EST sequence from *Physcomitrella patens*, IQD like protein (contig 5180) has been identified (Abel *et al.,* 2005), which can be a potential target for heavy metal stress tolerance (Fig 3). Although the deduced amino acid sequence appears to be truncated at the C- terminus (20 amino acid residue downstream of IQ67 domain) an appreciable similarity to the protein encoded by At1g01110 is found (33% identity) which includes the presence of MEME motif 3 at its N-terminus. Alignment of IQ67 domain of moss polypeptide reveals a deletion of six residues that correspond to the N-terminus of the second IQ67domain encoding exon of most *Arabidopsis* and *Rice* IQD proteins. As the IQ67 intron is in phase-0 and since both *A. thaliana* and *Oryza sativa* express an *IQD*-like gene in which the second IQ67 domain-encoding exon is replaced by an unrelated exon, it is unlikely that the contig 5180 DNA sequence is an artifact and probably represents either a novel variant of *IQD*-like genes or an ancestral gene of the *IQD* genes found in vascular plants (Abel *et al.,* 2005).

4.5. Calmodulin binding proteins in bryophytes

Plant response to a variety of environmental stresses is mediated in part by signaling processes involving cytosolic calcium and calcium binding protein, calmodulin (Knight *et al*., 1997; Plieth *et al.,* 1999; Sanders *et al.,* 1999). Genes encoding novel membrane transporter like proteins– Mcamb1, Mcamb2 have been identified in *Physcomitrella patens* (Takezawa and Minami, 2004). Calmodulin binds to Mcamb1 and Mcamb2 via interaction with basic

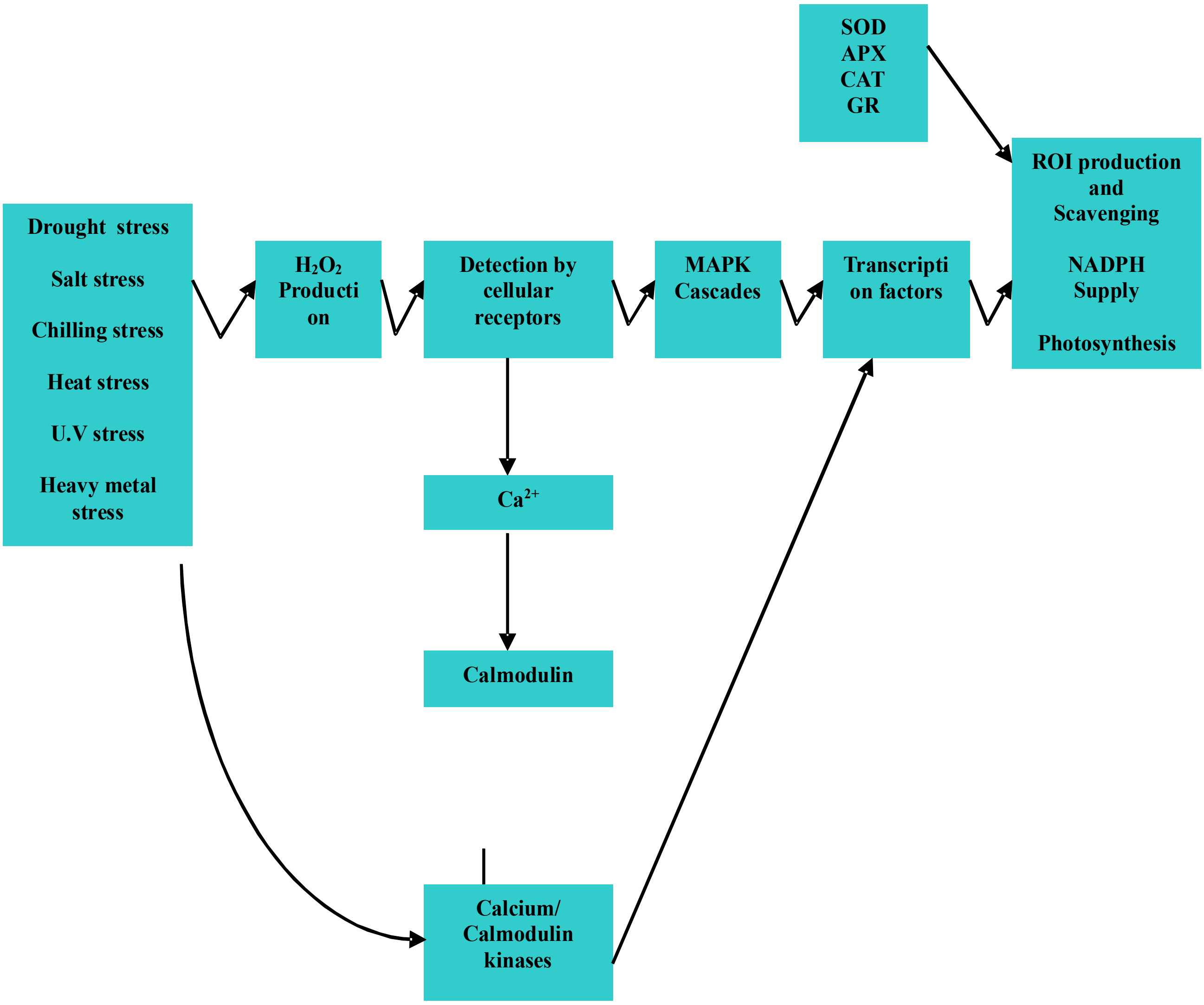

Fig. 3: Activation of signal transduction events during oxidative stress exhibiting potential nodes where cross talks can occur (Modified from Mittler, 2002)

amphiphillic amino acids in C-terminal domains. Levels of *Mcamb1* and *Mcamb2* transcripts increase dramatically following treatments with low temperature, hyperosmotic solute and stress hormone ABA. Calmodulin participates in cellular signaling events leading to increase in stress resistance through regulation of novel transporter like proteins (Takezawa and Minami, 2004). Hence, studies indicate that calcium might play a role in signaling events leading to the development of stress resistance through modulation of stress inducible CaM-binding transporters in bryophytes (Fig 3).

4.6. GC rich repetitive DNA sequences

Behaviour of repetitive DNA in response to lead and cadmium in two bryophytes namely *Leptodictyum riparium* and *Funaria hygrometrica* was studied (Bassi *et al.,* 2006). Metal treatment was accompanied by selective amplification of some GC-rich repetitive DNA sequences forming peculiar agglomerates inside the nucleus (Bassi *et al.,* 1995; Bassi, 1999). This amplification is quantitatively proportional to the time of exposure of the plants to metals and stops upon removal of metals from the culture medium. Results show that ribosomal DNA sequences are involved in this metal-induced repetitive DNA agglomerates formation (Bassi *et al.,* 2006). Thus focus should be on this particular fraction of the genome to identify the potential targets for stress tolerance in bryophytes.

4.7. Expansins

Expansins are extracellular proteins involved in cell wall loosening (Cosgrove, 2000). They play a definite role in plant growth and differentiation (caulonema formation). Expansins like proteins are reported in *Physcomitrella patens* (Schipper *et al.,* 2002). Both alpha (ά) and beta (β) expansins are present as gene families in this plant and expression analysis indicates that these genes respond to a complex regulation by hormonal and environmental factors. In particular, the expression of many expansins genes gets upregulated in stress conditions suggesting that they play a definite role in specific cellular differentiation displayed by *Physcomitrella patens* in response to such stresses. Six cDNA encoding expansins like proteins are found in *Physcomitrella patens*. PpExp1, PpExp2, PpExp and PpExp4 encode ά- expansins like proteins, whereas PpExpB1 and PpExpB2 encode β- expansins like proteins (Schipper *et al.,* 2002).

These provide a powerful tool to investigate the purported role of expansins in modulating plant growth and form via cell wall extensibility in response to stress conditions.

4.8. Messenger Ribonucleoprotein particles (mRNPs)

Alteration in gene expression elicited by stress is primarily regulated at the translational level (Oliver, 1991; Scott and Oliver, 1994; Oliver and Wood, 1997). The alteration in protein synthesis patterns during rehydration occurs as a result of differential selection of rehydrin mRNAs from a constant mRNA pool. mRNPs are proteins that permit restoration of protein synthesis following rehydration (Wood and Oliver, 1999). The studies on *Tortula ruralis* indicate that in mosses, the selection of rehydrin mRNA is in part aided by storage of these transcripts as messenger ribonucleoprotien particles (mRNPs) during drying.

Tr288 is a rehydrin in *Tortula ruralis* that codes for a protein having structural similarity to higher plants LEA proteins (Wood and Oliver, 1999). This rehydrin has a transcript that is abundant during slow drying and accumulates in polysomal fractions of cell extracts from slow dried gametophytes. The polysomal retention of Rps3a and Rps14, Rps16 and Rp123 transcripts in desiccated moss gametophyte indicates that not all transcripts made in response to a stress event are required for immediate use but may be synthesized and stored for the recovery period (Wood *et al.,* 2000). The formation of mRNPs in response to water loss and their possible roles in mRNA storage and protection has important consequences for the study of vegetative desiccation tolerance and perhaps stress responses of plants in general.

The ability to store components during any stress event that are needed for recovery offers a new dimension to the concept of damage control and rapid return to growth than does relatively slower activation and transcription of specific stress recovery genes thus making mRNPs promising tools for stress tolerance in bryophytes (Wood and Oliver, 1999).

Various field and culture studies have been carried out using bryophytes as heavy metal monitoring tools (Kuik and Wolterbeek, 1995; Markert *et al.,* 1996; Sucharova and Suchara 1998; Bargagli *et al.,* 2002; Schroder and Pesch 2005; Tyagi *et al.,* 2007). But, information on the relationship between heavy metal stress on bryophytes at biochemical, protein and gene level is rather incomplete.

5. Recommendations

Heavy metal pollution is a fast growing environmental problem, which calls for immediate attention. Owing to the higher cost outlay, inability to detect short lived deleterious effects and minute quantities of pollutants below the detection limits of conventional remediation technologies, the need of the hour is to use plants for environmental cleanup. Bryophytes qualify as promising candidates for such studies as they need minimum care during their life cycle. Surprisingly, potential of these plants has always been underestimated. Heterotrimeric guanine nucleotide binding proteins (G-proteins) are well characterized signaling molecules that interact with plasma membrane localized G-protein coupled receptors (GPCRs) and transduce majority (~80%) of extracellular signals across the cell membranes. However, these functions are believed to be performed by regulator of G-protein signaling (RGS) in plants. The main objective of this review is to provide description of the relevant molecules that might play leading roles in heavy metal stress tolerance in bryophytes. There are several processes including signal transduction, transcript directive, homeostasis and biosynthesis defense proteins which need to be thoroughly addressed to understand the stress tolerance mechanism in bryophytes. We, hope to promote the exploration of bryophytes at biochemical, protein and gene level as tools for combating heavy metal pollution.

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