Review Article

Glutathione as a Crucial Modulator of Phytohormone Signalling During Pathogen Defence in Plants

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Plant’s resistance to different stress factors is regulated by a complex signalling network which connects the individual signalling pathways, enabling them to fine tune their defence response. For more than last two decades, glutathione (GSH) is gradually gaining importance as a crucial player in this network. The present review summarizes the central role of GSH in modulating plant’s defence response to biotic stress, specially emphasizing the molecular mechanism of these regulations. Several transgenic approaches to constitutively enhance GSH levels have been followed and in most cases, these transgenic plants exhibited enhanced biotic stress tolerance. The post 2000 era envisaged a mechanistic approach in this field and GSH has been shown to modulate the defence signalling network by cross-communication with several stress-related phytohormones. GSH imparts stress tolerance against biotrophic infection via NPR1-dependent salicylic acid (SA) mediated pathway. GSH regulates SA accumulation at the level of isochorismate synthetase 1 (ICS1) expression and can also act in NPR1-independent pathway. A synergistic GSH-ethylene (ET) interplay during necrotrophic infection has also been reported. It has been demonstrated that GSH induces ET biosynthesis by modulating transcriptional and post-transcriptional regulations of its key enzymes. The cross-talk of GSH with jasmonic acid (JA) and abscisic acid (ABA) in alleviating stress has been reported as well. However, mechanistic details of the interaction between GSH and JA or ABA signaling pathways are not elucidated in details.

Keywords: Glutathione; Phytohormone Signalling; Pathogen Defence; Salicylic Acid; Ethylene; Jasmonic Acid

Introduction

Plants in their natural environment are continuously being threatened by a range of stress factors, including invasion by microbial pathogens, herbivorous insects as well as various abiotic stress conditions. Being immobile, plants have to respond to each of these attackers in a rapid and effective way in order to ensure survival. Plant’s resistance to different stress factors is a multifaceted regulatory network which links the various signalling pathways thus enabling them to fine tune their defence responses. Previous studies also envisaged that plant’s responses to various stress factors are regulated by multiple signalling pathways. A perfect synchronization of these pathways switches on the transcription of appropriate defence related genes and their downstream machinery ultimately helping the system to tide over unfavourable conditions. It has been well-documented that an interconnecting signalling network, comprising the salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) mediated signalling pathways, constitute the basic defence response strategy in plants (Glazebrook, 2005; Klessig et al., 2000; Loake and Grant, 2007; Pieterse et al., 2009; Thomma et al., 1998; van Loon et al., 2006). Glutathione (GSH; \(\gamma\)-glutamylcysteinyl glycine) is a low molecular weight non-protein tripeptide which is found in nearly all prokaryotic as well as eukaryotic cells. GSH represents the major pool of non-protein reduced

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sulphur and plays important roles in cell function and metabolism (Kunert and Foyer, 1993). It is gradually gaining importance as a vital player in this network and plays critical roles in combating biotic and abiotic stresses (Ghanta and Chattopadhyay, 2011).

This tripeptide thiol is a multifunctional metabolite and has drawn significant attention due to its wide distribution in most living organisms, abundance, broad redox potential and unique structural properties. The stability and ability of GSH to function as a cellular reductant has been derived from the single thiol group in the central cysteine. Along with ascorbate, GSH is considered as one of the most abundant redox couples in plant cells (May et al., 1998). It has largely been reported for more than two decades that GSH plays a crucial role in cellular processes including development, growth and adverse environmental responses in plants. Abiotic stresses like extreme temperatures, heavy metal contaminated soils, drought, salinity or air pollutants lead to the generation of reactive oxygen species (ROS) and alter the stability and ability of GSH to function as a cellular reductant. The ascorbate-GSH cycle that eliminates peroxides plays an important role in this respect (Noctor and Foyer, 1998). GSH also participates in the detoxification of a range of xenobiotics, herbicides, heavy metals and air pollutants like sulphur dioxide and ozone (Grill et al., 1989; May et al., 1998). GSH plays a critical role in biotic stress and its management as well (Dubreuil-Maurizi and Poinssot, 2012; Parisy et al., 2007). In fact, a direct link between the biosynthesis of GSH and the expression of defence related genes has been reported in Arabidopsis (Ball et al., 2004). Though the participation of GSH in plant responses to stress has long been reported (Dron et al., 1988; Wingate et al., 1988), yet the underlying molecular mechanism is still being explored.

**GSH as a Central Regulator in Biotic Stress Tolerance**

It has been demonstrated that significant changes in the GSH levels occur in the cells adjacent to the site of attempted pathogen ingress. This altered GSH levels then play an important role in regulating the induced defence response including the expression of genes such as glutathione-S-transferases (GSTs) (Jabs et al., 1996; Levine et al., 1994; Mauch and Dudler, 1993) and glutathione peroxidases (GPX) (Levine et al., 1994). Incompatible plant-pathogen interactions generate reactive oxygen species (ROS) and other products of lipid peroxidation which are detoxified by both GST and glutathione reductase (GR). It has been reported that during compatible barley-barley powdery mildew interactions activation of various antioxidative enzymes like GSTs and the ascorbate-GSH cycle occur presumably to lessen the detrimental effects of oxidative stress. On the other hand, these antioxidative reactions are either not activated or are only slightly activated in case of incompatible interactions (El-Zahaby, 1995). In general, pathogen invasion leads to enhanced accumulation of GSH in the cells which also increases the GSH/GSSG ratio and ultimately switches on the downstream defence related signalling cascade.

A transgenic approach to overexpress different GSH related genes in plants to constitutively enhance the GSH levels has widely been followed (Gomez et al., 2004; Gullner et al., 2001; Noctor et al., 1998; Xiang et al., 2001; Zhu et al., 1999). In most cases, these transgenic plants exhibited enhanced stress tolerance. While most of these transgenic plants displayed no phenotypic abnormalities, some suggested a variation. In one such study, transgenic tobacco plants were found to develop severe necrosis due to a hypersensitive response (HR) by overexpressing the γ-ECS enzyme (Creissen et al., 1999). This is perhaps because an excessive accumulation of GSH in the tissue led to oxidative stress. It has been reported that oxidative stress of intracellular origin can trigger HR and the excessive GSH redox potential (or GSSG accumulation) may lead to the activation of genetically programmed cell suicide pathways in the transgenic plants. This explanation is further supported by the fact that intracellular oxidative stress associated with GSSG accumulation can trigger the HR-like lesion formation (Chamnongpol et al., 1998; Smith et al., 1984; Willekens et al., 1997).

The A. thaliana cad2-1 and rax1-1 mutants have mutations in the GSH biosynthesis enzyme. Ball et al., (2004) reported that a changed GSH metabolism in these mutants leads to an alteration in the expression of 32 stress responsive genes. Another A. thaliana camalexin deficient mutant, pad2-1, was
isolated in the 1900s and was demonstrated to be susceptible to *Pseudomonas syringae* as well as *P. brassicae* infections (Glazebrook and Ausubel, 1994; Glazebrook et al., 1997). About a decade later, it was demonstrated that the disease susceptibility of this mutant was not due to camalexin deficiency but due to a mutation in the GSH biosynthetic pathway gene and consequent GSH depletion (Parisy et al., 2007). The *pad2-1* mutant again displayed decreased resistance to the feeding of insect larvae and this effect has been linked to decreased accumulation of glucosinolates (Schlaeppi et al., 2008). This susceptibility can be rescued by supplementation with exogenous GSH but not with other disulphide reductants like dithiothreitol (Schlaeppi et al., 2008).

It has subsequently been reported that formation of the glucosinolatethioglucose moiety encompasses a GSH-conjugate intermediate which is metabolized by a γ-glutamyl peptidase, GGP (Geu-Flores et al., 2009). Also, this GSH depleted mutant is deficient in camalexin because camalexin synthesis requires GSH as a precursor (Böttcher et al., 2009; Su et al., 2011).

In fact, synthesis of both camalexin and glucosinolates involve formation and metabolism of GSH-conjugate

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<tr>
<th>Pathogen</th>
<th>Host plant</th>
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<tr>
<td><em>Fusarium oxysporum</em></td>
<td><em>Cucumis melo</em> and <em>Lycopersicon esculentum</em></td>
<td>Bolter et al., 1993</td>
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<td><em>Drechslera avenae</em> and <em>Drechslera siccans</em></td>
<td><em>Avena sativa</em></td>
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<td><em>Erysiphe graminis</em></td>
<td><em>Triticum aestivum</em></td>
<td>Mauch and Dudler 1993</td>
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<td><em>Erysiphe graminis</em></td>
<td><em>Hordeum vulgare</em></td>
<td>El-Zahaby 1995</td>
</tr>
<tr>
<td><em>Cladosporium fulvum</em></td>
<td><em>Lycopersicon esculentum</em></td>
<td>May et al., 1996</td>
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<tr>
<td>Tobacco mosaic virus</td>
<td><em>Nicotiana tabacum</em></td>
<td>Fodor et al., 1997</td>
</tr>
<tr>
<td>*Pseudomonas syringae and <em>P. brassicae</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Glazebrook and Ausubel 1994; Glazebrook et al., 1997; Ball et al., 2004; Parisy et al., 2007; Mhamdi et al., 2010</td>
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<td><em>Alternaria brassicicola</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Van Wees et al., 2003</td>
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<td><em>Spodoptera littoralis</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Schlaeppi et al., 2008</td>
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<td><em>Pseudomonas syringae</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Chaouch et al., 2010</td>
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<tr>
<td><em>Phytophthora brassicae</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Roetschi et al., 2001; Maughan et al., 2010</td>
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<td>RNA viruses</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Wang et al., 2011</td>
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<tr>
<td><em>Pseudomonas syringae</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Ghanta et al., 2011</td>
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<td><em>Meloidogyne incognita</em></td>
<td><em>Medicago trunculata</em></td>
<td>Baldacci-Cresp et al., 2012</td>
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<tr>
<td><em>Phytophthora cinnamomi</em></td>
<td><em>Eucalyptus</em></td>
<td>Dempsey et al., 2012</td>
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<td><em>Botrytis cinerea</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Simon et al., 2013</td>
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<tr>
<td><em>Pseudomonas syringae</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Mhamdi et al., 2013</td>
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<td><em>Caterpillar herbivory</em></td>
<td><em>Arabidopsis thaliana</em></td>
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<td><em>Botrytis cinerea</em></td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>Kuzniak et al., 2013</td>
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<td><em>Colletotrichum gloeosporioides and Ralstonia solanacearum</em></td>
<td><em>Arabidopsis thaliana</em></td>
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<td><em>Alternaria alternata</em></td>
<td><em>Meniga arvensis</em></td>
<td>Sinha et al., 2013</td>
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<tr>
<td><em>Pseudomonas syringae</em> and <em>Botrytis cinerea</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Ghanta et al., 2014</td>
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<tr>
<td><em>Pseudomonas syringae</em> pv lachrymans</td>
<td><em>Cucumis sativus</em></td>
<td>Kuzniak et al., 2014</td>
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<tr>
<td><em>Magnaporthe oryzae</em></td>
<td><em>Oryza sativa</em></td>
<td>Zhang et al., 2015</td>
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<tr>
<td><em>Pseudomonas syringae</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Datta and Chattopadhyay 2015</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>Ferrari et al., 2003; Datta et al., 2015</td>
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</table>
which is hampered under low GSH conditions (Geu-Flores et al., 2011). This explains why the pad2-1 mutant displays constitutive camalexin deficiency and lower glucosinolates induction.

The proteins involved in imparting disease susceptibility of the GSH depleted pad2-1 mutant to P. syringae infection has been reported recently. It has been identified that the mutant actually fails to efficiently regulate several proteins involved in the PTI-related first line of defence as well as ETI-related R-gene products, thus signifying the dynamic role of GSH in plant defence (Datta and Chattopadhyay, 2015). These studies cumulatively suggest that the accumulation of GSH is essential for disease resistance. Using high-resolution imaging techniques the temporal and spatial changes of subcellular GSH level during Botrytis cinerea infection in A. thaliana have been shown (Simon et al., 2013). Another recent study demonstrated that increased GSH contributes to stress tolerance and global translational changes in A. thaliana. The translatome analysis also identified several novel genes related to auxin, ABA, and JA biosynthetic pathways as well as signalling genes whose transcription is induced in response to exogenous GSH treatment, which was not reported in any previous transcriptomic data (Cheng et al., 2015). Table 1 summarizes the different host-pathogen systems in which the role of GSH in imparting disease resistance has already been established.

**GSH and its Cross-Talk with Phytohormones During Biotic Stress**

Phytohormones are small molecules that are indispensable for the regulation of plant growth, development, reproduction, and survival. Diverse small-molecule phytohormones viz., SA, JA, ET, abscisic acid (ABA) and brassinosteroids play pivotal roles in regulating the plant defence signalling network (Dong, 1998; Dahl and Baldwin, 2007; Grant and Lamb, 2006; Howe and Jander, 2008; Loake and Grant, 2007; Pieterse et al., 2009; van Wees et al., 2003; van Loon et al., 2006; Vlot et al., 2008). Extensive synergistic and/or antagonistic cross-communications among their signalling pathways enables the plant to finely regulate its immune response (Bostock, 2005; Kunkel and Brooks, 2002; Pieterse et al., 2009; Reymond and Farmer, 1998). Although the detailed mechanisms of these cross-talks are not fully described as yet, accumulating evidence points to imperative roles for GSH in phytohormone signalling during biotic stress (Ghanta et al., 2014; Mhamdi et al., 2013; Spoel and Loake, 2011). The different milestones attained in exploring the role of GSH-phytohormone cross-talk in plant defence signalling network has been summarized in Table 2.

**GSH-SA Interplay**

Plants synthesize SA in response to invasion by a diverse range of phytopathogens and it plays an essential role in establishing both local and systemic acquired resistance (SAR) (Loake and Grant, 2007). SA signalling is mediated by at least two mechanisms, the NPR1 dependent and the NPR1 independent pathways (Blanco et al., 2009). Under normal condition, NPR1 exists as an oligomer. SA induces a change in the cellular redox potential, which leads to the reduction of NPR1 oligomer to its active monomeric form. It has been reported that the oligomerization of NPR1 is facilitated by its S-nitrosylation at cysteine-156 residue by S-nitroso glutathione (GSNO). Conversely, the SA-induced monomerization of the NPR1 oligomer is catalyzed by thioredoxins (TRXs) through reduction or oxidation of its intermolecular disulphide bonds (Tada et al., 2008). Monomeric NPR1 is then translocated from the cytosol into the nucleus where it acts as a transcriptional co-activator of several SA-responsive genes (Després et al., 2003; Mou et al., 2003). NPR1 is also a key molecule in modulating the SA-JA cross-talk during stress. In the cytosol, monomeric NPR1 negatively regulates JA-responsive gene expression, perhaps by inhibiting positive regulators of JA-responsive genes or by enabling the delivery of negative regulators of JA-responsive genes to the nucleus (Spoel et al., 2003).

Fascinatingly, the interplay of SA with ROS and GSH under various stress conditions has been supported by different lines of evidence (Herrera-Vásquez et al., 2015). One of the earliest reports suggesting GSH-SA interplay comes from a study on pea seedlings. In pea seedlings, a rise in the reduced GSH content and fall in the oxidized glutathione (GSSG) level, together with an increased GSH:GSSG ratio has been observed in response to exogenous SA treatment which signifies a modulation of GSH metabolism by SA (Srivastava and Dwivedi, 1998).
### Table 2: Different milestones attained in exploring the role GSH-phytohormone cross-talk in plant defence signalling network

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<thead>
<tr>
<th>Milestones</th>
<th>Host system</th>
<th>References</th>
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<tr>
<td>Exogenous SA treatment leads to an increase in GSH</td>
<td>Pea seedlings</td>
<td>(Srivastava and Dwivedi 1998)</td>
</tr>
<tr>
<td>SA induces GST transcription during plant defence via an ocs enhancer element in the GST promoter region</td>
<td>Arabidopsis</td>
<td>(Chen and Singh 1999)</td>
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<tr>
<td>Thermo-tolerance as a consequence of SA treatment coincides with an increase in GSH level but GSH:GSSG ratio remains unaltered</td>
<td>Tobacco</td>
<td>(Dat et al., 2000)</td>
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<tr>
<td>INA (SA analogue) treatment increases the GSH level leading to a reduction of NPR1 and subsequent PR1 gene expression</td>
<td>Arabidopsis</td>
<td>(Mou et al., 2003)</td>
</tr>
<tr>
<td>Exogenous treatments with SA, INA as well as pathogen infection increases GSH content and GSH:GSSG ratio</td>
<td>Arabidopsis; Tobacco-TMV infection</td>
<td>(Fodor et al., 1997; Vanacker et al., 2001; Mateo et al., 2006)</td>
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<tr>
<td>Constitutive overexpression of SA induces GSH-mediated nickel tolerance</td>
<td><em>Thlaspi</em> sp.</td>
<td>(Freeman et al., 2005)</td>
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<tr>
<td>Protection of ozone-induced leaf injury by SA coincides with an increase in <em>de novo</em> GSH synthesis</td>
<td>Arabidopsis</td>
<td>(Yoshida et al., 2009)</td>
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<tr>
<td>GR1-dependent GSH statusplays a crucial role in leaf responses to intracellular H$_2$O$_2$ including accumulation of SA, induction of PR genes and SA signalling pathway</td>
<td>Arabidopsis</td>
<td>(Mhamdi et al., 2010)</td>
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<tr>
<td>Higher GSH level observed in SA-deficient plants during RNA virus infection</td>
<td>Arabidopsis-CMV</td>
<td>(Wang et al., 2011)</td>
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<tr>
<td>GSH regulates the SA-mediated suppression of JA signalling</td>
<td>Arabidopsis-Alternaria brassicicola/Botrytis cinerea</td>
<td>(Koornneef et al., 2008)</td>
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<tr>
<td>JA treatment decreases GSH level</td>
<td>Arabidopsis</td>
<td>(Spoel and Loake 2011)</td>
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<tr>
<td>Simultaneous SA and JA application increase GSH level suggesting a prioritization of the SA pathway</td>
<td>Arabidopsis</td>
<td>(Koornneef et al., 2008)</td>
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<tr>
<td>GSH signaling acts through NPR1-dependent SA-mediated pathway to mitigate biotic stress</td>
<td>Tobacco</td>
<td>(Ghanta et al., 2011b)</td>
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<tr>
<td>GSH regulates SA accumulation at the level of ICS1expression and GSH also act independently of NPR1 to allow increased intracellular H$_2$O$_2$ to activate SA signalling</td>
<td>Arabidopsis</td>
<td>(Han et al., 2013a)</td>
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<td><strong>GSH-ET interplay</strong></td>
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<tr>
<td>An ET-responsive GST gene cluster was characterised in carnation</td>
<td>Carnation</td>
<td>(Itzhaki and Woodson 1993)</td>
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<td>ET synthesis is hampered when the GSH pool shifts towards oxidized state by exogenous GSSG treatment</td>
<td>White spruce</td>
<td>(Belmonte et al., 2005)</td>
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<td>ACO transcripts increased in spruce somatic embryos grown in excess GSH condition</td>
<td>White spruce</td>
<td>(Stasolla et al., 2004)</td>
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<tr>
<td><em>S</em>-adenosylmethionine synthase transcript decreases in <em>Brassica napus</em> grown in excess GSSG condition</td>
<td><em>Brassica</em></td>
<td>(Stasolla et al., 2008)</td>
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<tr>
<td>ET biosynthesis is assumed to be controlled by GSH via transcriptional regulation of ACO and <em>S</em>-adenosylmethionine synthase</td>
<td><em>Brassica</em>; Spruce</td>
<td>(Stasolla 2010)</td>
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<tr>
<td>ET controls GSH biosynthesis positively in ozone exposed <em>A. thaliana</em> leaves</td>
<td>Arabidopsis</td>
<td>(Yoshida et al., 2009)</td>
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<tr>
<td>GSH-dependent lead resistance was impaired in ET signalling mutant, ein2-1</td>
<td>Arabidopsis</td>
<td>(Cao et al., 2009)</td>
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<tr>
<td>Multistep involvement of GSH with SA and ET to combat stress</td>
<td>Tobacco-Alternaria alternata</td>
<td>(Ghanta et al., 2014)</td>
</tr>
<tr>
<td>GSH induces ET biosynthesis by modulating the transcriptional and post-transcriptional regulations of its key enzymes, ACS and ACO</td>
<td>Arabidopsis-Botrytis cinerea/salt stress</td>
<td>(Datta et al., 2015)</td>
</tr>
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</table>
Subsequently, SA and H$_2$O$_2$ were shown to induce GST transcription during plant defence via an ocs element which is an enhancer element present in the GST promoter region (Chen and Singh, 1999). In another study, thermo-tolerance as a consequence of SA treatment was shown to coincide with an increase in both GSH and GSSG levels in shoot while the GSH redox ratio remained unaltered (Dat et al., 2000).

A treatment with 2, 6-dichloroisonicotinic acid (INA), the biologically active analogue of SA, augmented the GSH level in cells leading to the reduction of NPR1 and subsequent expression of the PR1 gene (Mou et al., 2003). Similarly, exogenous treatments with SA, INA as well as pathogen infection have been reported to increase GSH content and GSH: GSSG ratio in plants (Fodor et al., 1997; Mateo et al., 2006; Vanacker et al., 2001). In several reports, changes in GSH levels were reported to occur during salinity as well as osmotic stresses (Borsani et al., 2001). In another study, GSH-mediated nickel tolerance was shown be induced due to constitutive overexpression of SA in Thlaspi hyper accumulators (Freeman et al., 2005). Protection of ozone-induced leaf injury in A. thaliana by SA coincided with an increase in the de novo synthesis of GSH (Yoshida et al., 2009). In fact, ozone exposure leads to ROS generation and subsequent cell death. SA and ET production is induced under such condition to decrease the magnitude of ozone-induced cell death via induction of GSH biosynthesis. It has been reported that unlike the wild-type, mutants deficient in ET signalling (ein2) or SA biosynthesis (sid2) generated high levels of superoxide, lower levels of GSH and exhibited visible leaf injury. The activities of the GSH biosynthetic enzymes were also affected in these mutants. Furthermore, ozone-induced leaf damage detected in ein2 and sid2 was alleviated by exogenous GSH treatment. GSH status has been demonstrated to regulate SA and other biotic stress response pathways in A. thaliana. It has been demonstrated that the GR1-dependent GSH statusplays a crucial role in modulating multiple leaf responses to intracellular H$_2$O$_2$ including limitation of lesion formation, SA accumulation, induction of PR genes and signalling through SA and JA pathways (Mhamdi et al., 2010). In another report, the role of GSH against RNA viruses in SA-deficient plants has been described. The levels of virus replication were found to be higher in the SA-deficient plants during the first 10 days, but lower than the wild-type seedlings 20

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<tr>
<th><strong>GSH-JA interplay</strong></th>
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<tr>
<td>JA treatment increases GSH synthesis but did not alter the GSH content in unstressed plants</td>
<td>Arabidopsis</td>
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<tr>
<td>JA increases GSH metabolism under water stress</td>
<td>Agropyron cristatum</td>
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<td>Intracellular GSH is involved in methyl MeJA signalling</td>
<td>Arabidopsis</td>
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<tr>
<td>GSNOR mediates JA and ET biosynthesis and JA-elicited responses in in response to insect feeding</td>
<td>Arabidopsis</td>
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<td>MeJA treatment induces GPX expression</td>
<td>Arabidopsis</td>
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<td>Increased GSH confers tolerance to drought and salt stress by enhancing global translation of JA-responsive genes</td>
<td>Arabidopsis</td>
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<tr>
<td>GSH modulates the antagonistic interaction between SA and JA pathways at the level of NPR1</td>
<td>Arabidopsis-Pseudomonas syringae</td>
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<th><strong>GSH-ABA Interplay</strong></th>
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<tr>
<td>In two maize genotypes differing in stress tolerance, ABA differentially affected the GSH content, GSH:GSSG ratio, GR activity, and g-ECS transcript level</td>
<td>Maize</td>
</tr>
<tr>
<td>GSH content did not vary in potato tubers treated with ABA</td>
<td>Potato</td>
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<td>GSH-ABA interplay provides stress tolerance against abiotic stress factors</td>
<td>Arabidopsis; Wheat</td>
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<th><strong>GSH-SA interplay</strong></th>
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<tr>
<td>Exogenous SA treatment leads to an increase in GSH</td>
<td>Pea seedlings</td>
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days after infection. It has been demonstrated that the higher level of GSH and ascorbic acid observed in SA-deficient plants perhaps contribute to their alleviated symptoms (Wang et al., 2011).

GSH has also been shown to interfere with SA-mediated suppression of JA signalling in plants. The boost in GSH levels after SA treatment was shown to coincide accurately with the window of opportunity in which SA could suppress JA-induced PDF1.2 expression. Inhibition of GSH biosynthesis by l-buthioninesulfoximine (BSO) strongly reduced this suppression of the JA-responsive PDF1.2 gene by SA (Koornneef and Pieterse, 2008). Interestingly, JA can also influence the redox state of cells by decreasing the total amount of GSH and this shifts the GSH:GSSG ratio towards the oxidized state (Spoel and Loake, 2011). However, on simultaneous application of SA and JA, an increase in the GSH level was observed suggesting a prioritization of the SA pathway over the JA pathway (Koornneef et al., 2008). Nonetheless, the mechanism of how does the SA-mediated cellular reduction modulates JA-inducible responses still remains obscure.

To obtain deeper insight into the mechanism how the GSH-SA interplay is involved in mitigating biotic stress, Ghanta et al. (2011b) used transgenic tobacco plants with enhanced GSH content and exhibiting resistance to pathogenesis as well. It was observed that the expression levels of NPR1 and NPR1-dependent genes like PR1, mitogen-activated protein kinase kinase, glutamine synthetase, etc. were significantly enhanced in the transgenic plants as compared to the wild-type. Conversely, no significant alteration in the expression levels of NPR1-independent genes like PR2, PR5, and short-chain dehydrogenase/reductase family protein was observed. These observations suggested that GSH activates SA-mediated defence, presumably through the NPR1-dependent pathway (Ghanta et al., 2011b; Ghanta and Chattopadhyay, 2011). In a subsequent study, the transgenic tobacco plants with enhanced GSH levels were reported to synthesize more SA than the wild-type plants (Ghanta et al., 2011a). Activation of SA related genes and enhanced resistance to pathogenesis under augmented GSH condition was also observed in later studies (Ghanta et al., 2014).

It has subsequently been reported that GSH regulates SA accumulation at the level of isochorismate synthetase 1 (ICS1) expression and that an increase in the intracellular H$_2$O$_2$ level can function to activate the SA signalling (Han et al., 2013a). The H$_2$O$_2$-triggered changes in GSH status have been suggested not merely to be a passive response to oxidative stress. In fact, this modulation of GSH status links the elevated intracellular H$_2$O$_2$ production to activation of the ICS1-dependent SA pathway. The accumulated SA then leads to activation of NPR1 function through reductive processes, to which GSH also contributes. This general model has been depicted in Fig. 1.

**Fig. 1:** GSH induces SA signalling via NPR1 dependent and NPR1 independent pathways in plants. GSH on one hand directly induces NPR1 dependent pathway presumably through thioredoxins and glutaredoxins. On the other hand, oxidation by H$_2$O$_2$ modulates GSH status. This oxidative modulation is a part of the signal network required for optimal ICS1 dependent SA accumulation that then leads to activation of NPR1 dependent as well as independent pathways.

**GSH-ET Interplay**

ET, a gaseous phytohormone, is known to play significant roles in plant defence and participate in cross-talk with other signalling molecules. ET and JA have been widely reported to act synergistically against necrotrophic pathogens (Penninckx et al., 1998). In some cases however, a synergistic interaction between ET and SA has also been demonstrated to induce SAR and SA-mediated gene expression in plants (Lawton et al., 1994; Verberne et al., 2003; Vos et al., 2006).
Reports demonstrating GSH-ET interplay dates back to the early 1990s when an ET-responsive GST gene cluster was characterized in carnation (Itzhaki and Woodson, 1993). In another report, ET synthesis was observed to be hampered in spruce somatic embryo when the GSH pool was shifted towards oxidized state by exogenous GSSG treatment (Belmonte et al., 2005). Molecular studies revealed that ACC oxidase (ACO) transcripts increased in spruce somatic embryos grown in excess GSH (reduced) condition (Stasolla et al., 2004). In a subsequent study, transcripts of S-adenosylmethionine synthase was found to be decreased in *Brassica napus* when grown in excess GSSG (oxidized) condition (Stasolla et al., 2008). Hence, the biosynthesis of ET has been assumed to be controlled by GSH via transcriptional regulation of the two biosynthetic enzymes: S-adenosylmethionine synthase and ACO (Stasolla, 2010).

ET has also been reported to control GSH biosynthesis positively in ozone exposed *A. thaliana* leaves (Yoshida et al., 2009). Again, the ein2-1 mutant with impaired ET signalling has been shown to exhibit impaired GSH-dependent lead resistance, which was related to constitutive repression of γ-ECS gene and consequently reduced GSH content (Cao et al., 2009). In transgenic tobacco with enhanced GSH content, up-regulation of ET related transcripts like ACO, ERF4 and WRKY1 and up-accumulation of proteins like ACC synthase (ACS) and ACO has been reported recently (Ghanta et al., 2014). These transgenic plants have also been reported to exhibit tolerance against pathogenesis as well as osmotic stress thus demonstrating the involvement of GSH-ET interplay in imparting stress tolerance in plants (Ghanta et al., 2014; Kumar et al., 2014).

The molecular mechanism of the GSH-ET cross-talk during necrotrophic infection as well as abiotic stress has been solved only very recently. It has been demonstrated that GSH induces ET biosynthesis by modulating the transcriptional and post-transcriptional regulations of its key enzymes, ACS and ACO in *A. thaliana* (Datta et al., 2015). Transgenic plants with enhanced GSH content were found exhibit up-regulation of ACS2, ACS6, and ACO1 at transcript as well as protein levels while down-regulation was observed in the GSH depleted *pad2-1* mutant. Furthermore, GSH was shown to induce ACS2 and ACS6 transcription in a WRKY33 dependent manner. For ACO1, however, GSH increased the stability of ACO1 mRNA without affecting its promoter activity. In addition, the ACO1 protein can be a subject for S-glutathionylation while S-glutathionylation of ACS2 and ACS6 proteins was not detected. Thus, a dual-level regulation of ET biosynthesis by GSH during stress has been proposed (Fig. 2; Datta et al., 2015).

**GSH - JA Interplay**

The role of JA in plant defence has long been reported (Farmer and Ryan, 1992; Gundlach et al., 1992; Turner et al., 2002). JA-dependent signalling has been reported to play a crucial role in pathogen attack, especially necrotrophs, wounding and insect feeding (Glazebrook, 2005; Thomma et al., 2001). JA has been known to function antagonistically with SA in defence signalling. JA treatment has been shown to increase the mRNA levels and the capacity for GSH synthesis but it did not alter the GSH content in unstressed plants (Xiang and Oliver, 1998). In a later study, it was observed that JA leads to an increase in GSH metabolism under water stress in *Agropyron cristatum* (Shan and Liang, 2010). Involvement of intracellular GSH has also been studied in methyl jasmonate (MeJA) signalling (Akteret et al., 2010). Again, S-Nitrosoglutathione reductase (GSNOR) has been shown to mediate JA and ET biosynthesis in *Nicotiana attenuata* in response to insect feeding (Wünsche et al., 2011). MeJA treatment has also been reported to induce expression of GPX in *Pueraria mirifica* (Saisavoey et al., 2014). It has recently been demonstrated that increased GSH confers tolerance to drought and salt stress in *Arabidopsis* by enhancing global translation of JA-responsive genes (Cheng et al., 2015). GSH has also been implicated in the antagonistic interaction between SA and JA pathways at the level of NPR1 as discussed earlier (Koornneef et al., 2008; Spoel et al., 2003).

Two possibilities could explain the dual effect of GSH on signalling through the SA and JA pathways in response to stress (Han et al., 2013b). First, GSH status can act to modulate a master switch that regulates the oxidative activation of both pathways. A second scenario would involve regulation of both pathways in parallel. Whatever may be the case, the SA- JA interplay mediated by other redox-linked factors could act downstream to determine the relative
GSH-ABA Interplay

Apart from these three phytohormones, ABA also plays a significant role in environmental stress tolerance. ABA cross-communicates with the SA-JA-ET network by antagonizing the onset of SA-dependent defences and SAR (Mohr and Cahill, 2007; Yasuda et al., 2008). Moreover, ABA attenuates the JA/ET-dependent gene expression (Anderson et al., 2004) and affects JA biosynthesis and resistance against JA-inducing necrotrophic pathogens (Adie et al., 2007; Flors et al., 2008). Interplay between ABA and GSH has also been reported. ABA has been demonstrated to differentially regulate the GSH content, GSH:GSSG ratio, γ-ECS transcript level and GR activity in two maize genotypes which varies in their stress tolerance potentials (Kellos et al., 2008). However, it was subsequently reported that ABA treatment did not alter the GSH levels in potato tubers (Stroiński et al., 2010). In several other studies, GSH-ABA interplay has been reported in providing stress tolerance against abiotic stress factors (Chen et al., 2012; Wei et al., 2015). But whether this interplay is also involved in imparting biotic stress tolerance remains to be studied in future.
Conclusion and Future Perspective

Dissecting the signalling network that operates during disease development in plants has revealed the association of various stress-related phytohormones along with several other signal molecules. GSH is mainly associated with the activation and modulation of different phytohormones and subsequent regulation of a various resistance genes. Most of the studies were focused on the cross-talk of GSH with SA, ET, and JA signalling pathways during stress. In SA and JA signalling pathways, GSH serves as an important intermediate to modulate their signalling cascades through NPR1 during specific stress responses. Again, in some cases, JA elicitation under stress condition can also induce GSH biosynthesis in plants. On the other hand, GSH enhances ET biosynthesis by a two-step process in response to necrotrophic infection as well as abiotic stress. Identification of the GSH-mediated induction of the ABA signalling pathway can provide a distinct linkage between biotic and abiotic stress responses as well. It can also add a more elaborate view of the interrelationship among various stress hormones during disease resistance in plants. However, the molecular mechanisms of the GSH-JA and GSH-ABA cross-talks during various stress responses are still unsolved. Whether GSH can induce the signalling cascade of these phytohormones or their biosynthesis pathways during stress needs to be investigated in future.

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