

## Glutathione signaling acts through NPR1-dependent SA-mediated pathway to mitigate biotic stress

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Glutathione (GSH) has widely been known to be a multifunctional molecule especially as an antioxidant up until now, but has found a new role in plant defense signaling. Research from the past three decades indicate that GSH is a player in pathogen defense in plants, but the mechanism underlying this has not been elucidated fully. We have recently shown that GSH acts as a signaling molecule and mitigates biotic stress through non-expressor of PR genes 1 (NPR1)-dependent salicylic acid (SA)-mediated pathway. Transgenic tobacco with enhanced level of GSH (*NtGB* lines) was found to synthesize more SA, was capable of enhanced expression of genes belonging to NPR1-dependent SA-mediated pathway, were resistant to *Pseudomonas syringae*, the biotrophic pathogen and many SA-related proteins were upregulated. These results gathered experimental evidence on the mechanism through which GSH combats biotic stress. In continuation with our previous investigation we show here that the expression of *glutathione S-transferase (GST)*, the NPR1-independent SA-mediated gene was unchanged in transgenic tobacco with enhanced level of GSH as compared to wild-type plants. Additionally, the transgenic plants were barely resistant to *Botrytis cinerea*, the necrotrophic pathogen. SA-treatment led to enhanced level of expression of *pathogenesis-related protein gene (PR1)* and *PR4* as against *short-chain dehydrogenase/reductase family protein (SDRLP)* and *allene oxide synthase (AOS)*. These data provided significant insight into the involvement of

GSH in NPR1-dependent SA-mediated pathway in mitigating biotic stress.

Plant responses to different environmental stresses are achieved through integrating shared signaling networks and mediated by the synergistic or antagonistic interactions with the phytohormones viz. SA, jasmonic acid (JA), ethylene (ET), abscisic acid (ABA) and reactive oxygen species (ROS).<sup>1</sup> Previous studies have shown that in response to pathogen attack, plants produce a highly specific blend of SA, JA and ET, resulting in the activation of distinct sets of defense-related genes.<sup>2,3</sup> Regulatory functions for ROS in defense, with a focus on the response to pathogen infection occur in conjunction with other plant signaling molecules, particularly with SA and nitric oxide (NO).<sup>4-6</sup> Till date, numerous physiological functions have been attributed to GSH in plants.<sup>7-11</sup> In addition to previous studies, recent study has also shown that GSH acts as a signaling molecule in combating biotic stress through NPR1-dependent SA-mediated pathway.<sup>12,13</sup>

Our recent investigation involved raising of transgenic tobacco overexpressing gamma-glutamylcysteine synthetase ( $\gamma$ -ECS), the rate-limiting enzyme of the GSH biosynthetic pathway.<sup>12</sup> The stable integration and enhanced expression of the transgene at the mRNA as well as protein level was confirmed by Southern blot, quantitative RT-PCR and western blot analysis respectively. The transgenic plants of the T<sub>2</sub> generation (Fig. 1), the phenotype of which was similar to that of wild-type plants were found to be capable

**Key words:** GSH, signaling molecule, biotrophic pathogen, NPR-1, PR-1, PR-4, transgenic tobacco

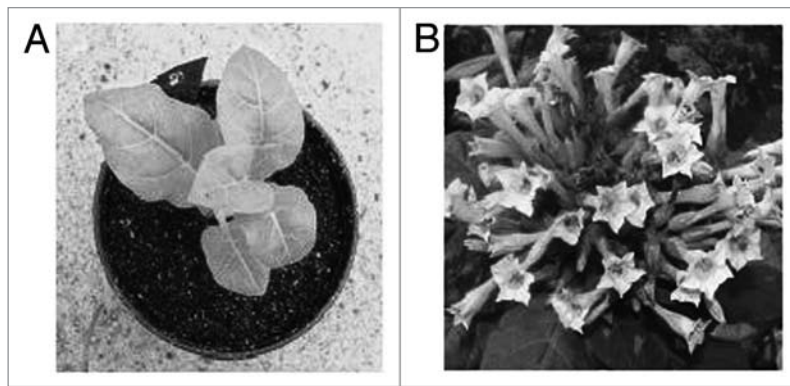
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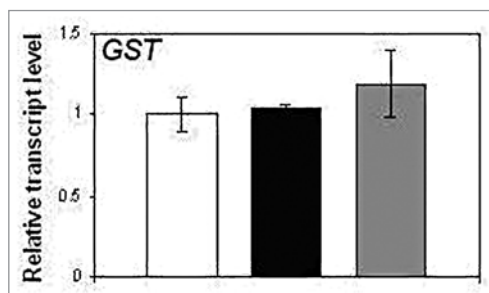
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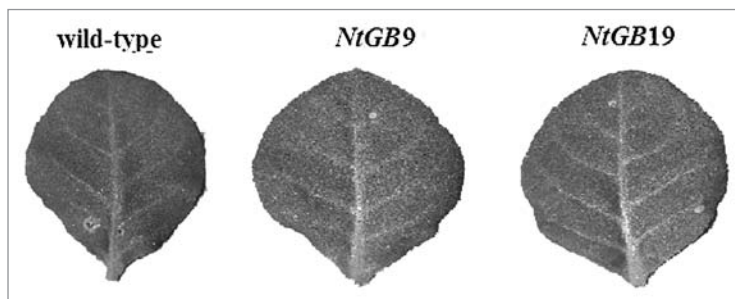
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**Figure 1.** Transgenic tobacco of  $T_2$  generation, (A) three-week-old plant, (B) mature plant.



**Figure 2.** Expression pattern of *GST* in wild-type and *NtGB* lines.



**Figure 3.** Resistance pattern of wild-type and *NtGB* lines against *Botrytis cinerea*.

of synthesizing enhanced amount of GSH as confirmed by HPLC analysis.

In the present study, the expression profile of *GST* was analyzed in *NtGB* lines by quantitative RT-PCR (qRT-PCR) and found that the expression level of this gene is unchanged in *NtGB* lines as compared to wild-type plants (Fig. 2). *GST* is known to be a NPR1-independent SA-related gene.<sup>14</sup> This suggests that GSH does not follow the NPR1-independent SA-mediated pathway in defense signaling.

Disease test assay with *NtGB* lines and wild-type plants was performed using *B. cinerea* and the *NtGB* lines

showed negligible rate of resistance to this necrotrophic pathogen (Fig. 3). SA signaling has been known to control defense against biotrophic pathogen in contrast, JA/ET signaling controls defense against necrotrophic pathogen.<sup>15</sup> Thus it has again been proved that GSH is not an active member in the crosstalk of JA-mediated pathway, rather it follows the SA-mediated pathway as has been evidenced earlier.<sup>12</sup>

Additionally, the leaves of wild-type and *NtGB* lines were treated with 1 mM SA and the expression of *PRI*, *SDRLP*, *AOS* and *PR4* genes were analyzed and

compared to untreated plants to simulate pathogen infection. The expression of *PRI* increased after exogenous application of SA. In case of *PR4*, the ET marker, the expression level increased in *NtGB* lines. On the other hand, the level of *SDRLP* was nearly the same. However, the expression of *AOS* was absent in SA-treated leaves (Fig. 4). *PRI* has been known to be induced by SA-treatment<sup>16</sup> which can be corroborated with our results. In addition, ET is known to enhance SA/NPR1-dependent defense responses,<sup>17</sup> which was reflected in our study as well. *AOS*, the biosynthetic pathway gene of JA, further known to be the antagonist of SA, was downregulated in SA-treated plants.

Taken together, it can be summarized that this study provided new evidence on the involvement of GSH with SA in NPR1-dependent manner in combating biotic stress. Additionally, it can be claimed that GSH is a signaling molecule which takes an active part in the cross-communication with other established signaling molecules like SA, JA, ET in induced defense responses and has an immense standpoint in plant defense signaling.

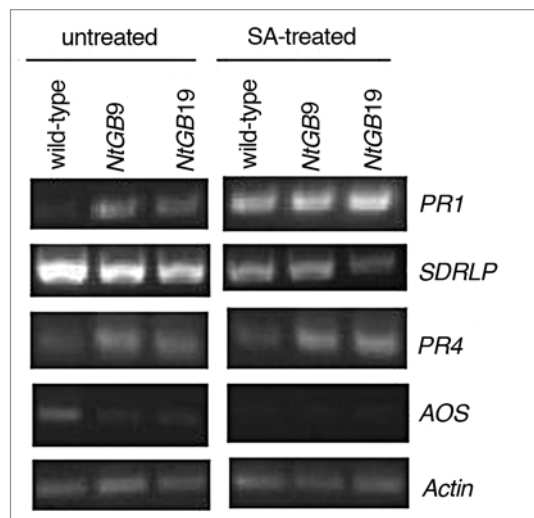
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**Figure 4.** Gene expression pattern of *PR1*, *SDRLP*, *PR4* and *AOS* in untreated and SA-treated wild-type and *NtGB* lines.