

Review

Sub-proteome S-nitrosylation analysis in *Brassica juncea* hints at the regulation of Brassicaceae specific as well as other vital metabolic pathway(s) by nitric oxide and suggests post-translational modifications cross-talk



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ARTICLE INFO

Article history:

Received 1 April 2014

Revised 21 August 2014

Available online 28 August 2014

Keywords:

Nitric oxide

Thiol pool

S-nitrosylation

Cold stress

Brassica juncea

ABSTRACT

Abiotic stress affects the normal physiology of the plants and results in crop loss. *Brassica juncea* is an oil yielding crop affected by abiotic stress. In future, over 30% yield loss by abiotic stress is predicted in India. Understanding the mechanism of plant response to stress would help in developing stress tolerant crops. Nitric oxide (NO) is now viewed as a remarkably important signaling molecule, involved in regulating stress responses. S-Nitrosylation is a NO based post-translational modification (PTM), linked with the regulation of many physiologically relevant targets. In the last decade, over 700 functionally varied S-nitrosylated proteins were identified, which suggested broad-spectrum regulation. To understand the physiological significance of S-nitrosylation, it was analyzed in cold stress. Functional categorization and validation of some of the *B. juncea* S-nitrosylated targets, suggested that NO produced during stress regulates cellular detoxification by modulating enzymes of ascorbate glutathione cycle, superoxide dismutase, glutathione S-transferase and glyoxalase I by S-nitrosylation in crude, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) depleted and apoplastic fractions. Interestingly, S-nitrosylation of enzymes associated with glucosinolate hydrolysis pathway, suggests a novel regulation of this Brassicaceae specific pathway by NO. Moreover, identification of enzymes of Glycolysis and Calvin cycle in crude and RuBisCO depleted fractions showed the regulation of metabolic as well as photosynthetic pathways by S-nitrosylation. S-Nitrosylation of cell wall modifying and proteolytic enzymes in the apoplast suggested differential and spatial regulation by S-nitrosylation. To have an overview of physiological role(s) of NO, collective information on NO based signaling (mainly by S-nitrosylation) is presented in this review.

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Abbreviations: Nitric oxide, NO; S-Nitrosothiols, SNOs; S-Nitrosoglutathione, GSNO.

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1. Introduction

Brassica juncea (Indian mustard) is an oil yielding crop of *Brassicaceae* family in the order Capparales. *B. juncea* faces yield loss due to abiotic stress (cold, salinity and heavy metal [1–3]). Although, India is the second largest cultivator of *Brassica* sp., but due to crop loss by these stress, it is not able to meet its annual edible oil requirement and 40% of its requirements are met by import [1]. Moreover, to further aggravate the situation, over 30% yield loss by abiotic stress is predicted in India alone. Therefore, it is important to understand its stress signaling mechanism, which would help in generating stress tolerant crops. In the last two decades, nitric oxide (NO) has emerged as an important signaling molecule, which regulates plant growth and development [4–6]. Its role in regulating large number of physiological processes and providing stress tolerance has been extensively reviewed [7–14]. Most of the work on NO signaling has been done on *A. thaliana* (a model member of *Brassicaceae*). However, the current status of NO signaling in crops including *B. juncea* has not been reviewed, therefore, this review presents evidences for NO signaling in *B. juncea* (Fig. 1). Additionally, effect of stress on the thiol pool is also described. As NO regulates diverse biological processes by regulating proteins through NO based post-translational modifications (PTMs) [15,16], current status of these PTMs in *B. juncea* is also presented. An attempt has also been made to understand the effect of NO in regulating both *Brassicaceae*

specific (glucosinolate-hydrolysis pathway) as well as other vital metabolic and regulatory pathways. Moreover, this review highlights the areas where further work is required to understand NO signaling in the Indian mustard particularly.

2. Stress induced nitric oxide production and thiol pool modulation

Eight major pathways, classified as oxidative and reductive pathways produce NO in plants [17–19]. Oxidative pathways include oxidation of L-arginine [by nitric oxide synthase (NOS)-like enzyme], polyamines and hydroxylamine, while reductive pathways include reduction of nitrite by enzymatic- [by nitrate reductase (NR), nitrite:NO reductase (Ni-NOR), xanthine oxidoreductase (XOR), cytochrome c oxidase and/or reductase] and non-enzymatic pathways (in an acidic environment). NOS-like enzyme catalyzes the oxidation of L-arginine to citrulline and NO is released as a by-product of the reaction. NOS-like enzyme activity showed 78.1% and 139.5% increase at 4 and 0 °C stress respectively in *Choripora bungeana* suspension cultures [20]. Additionally, treatment with L-NNA (N (omega)-nitro-L-arginine, an inhibitor of NOS) inhibited (70%) NO production in cold (30 days) treated *Solanum lycopersicum* fruits, therefore, indicating NOS-like enzyme dependent NO production in cold [21]. NR is the major NO producing enzymes and produce NO by reducing nitrite in the presence of NAD(P)H. Cold

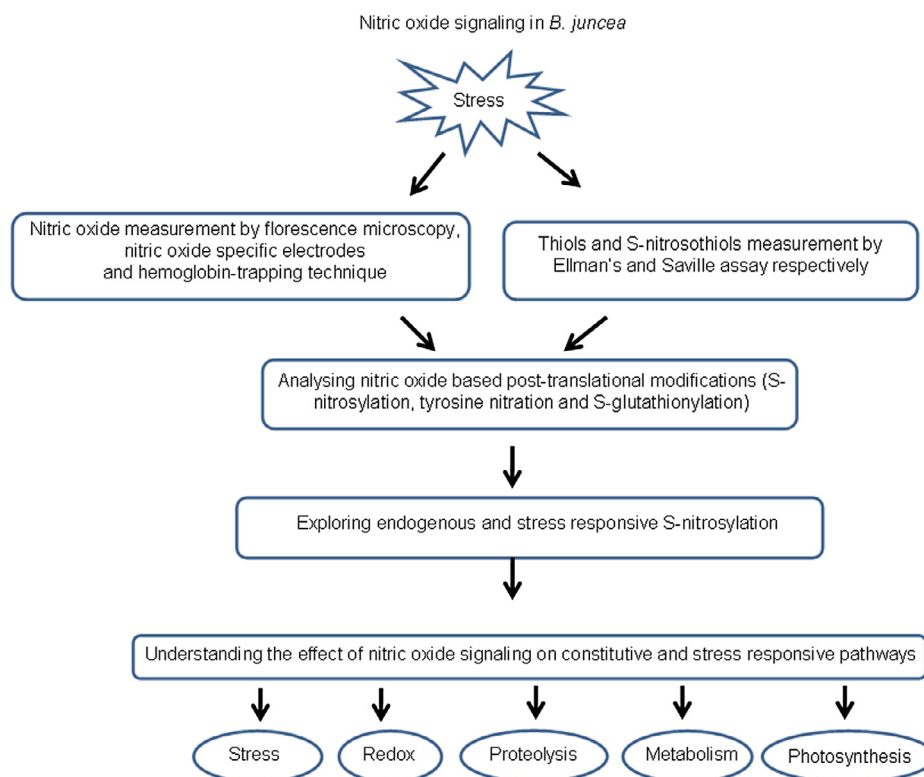


Fig. 1. Flow diagram showing steps of nitric oxide signaling analysis in *B. juncea*.

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