Bidens pilosa (Asteraceae)

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Bidens pilosa (Asteraceae) is an easy-to-grow medicinal herb which is also consumed as a leafy vegetable. This plant has been shown to have various health benefits and treatment of disease/conditions such as malaria, hypertension, obesity and typhoid. Interestingly, some secondary metabolites such as hydroxycinnamic acid derivatives that have been identified in *B. pilosa* have been shown to have various bioactivities health such as anti-cancer properties, HIV-integrase inhibition and anti-diabetic properties. In this study, exogenous treatment of *B. pilosa* leaves with two signal molecules (MeJA and MeSA) induced metabolic changes, differentially perturbating the accumulation of these biologically important metabolites. Moreover, the perturbation of isomeric molecules, especially the *cis* geometrical isomers of HCA derivatives by both treatments, further point to the biological significance of these molecules during physiological responses to stress. The results highlight the possibility of using phytohormones to enhance the accumulation of bioactive secondary metabolites in this plant.

Keywords: Bidens pilosa ; metabolomics ; elicitation ; caftaric acid ; chicoric acid ; chlorogenic acid ; flavonoids ; methyl jasmonate ; methyl salicylate ; phytohormones

1. Introduction

Bidens pilosa L. (*B. pilosa*) is a cosmopolitan weed occurring mostly in hot tropical areas including sub-Saharan Africa, where it is a widely consumed leafy green vegetable source [1][2]. This plant is not only recognized for its nutritional value, but also for its pharmacological and bio-medical importance [3][4]. A diverse group of important compounds have been identified in *B. pilosa* such as aliphatics, terpenoids, tannins, alkaloids porphyrins, and phenylpropanoids including hydroxycinnamic acids (HCAs) and derivatives, chalcone okanins and flavonoids [3][5][6][7]. Some phenylpropanoids that have been reported in *B. pilosa* include a wide range of chlorogenic acids (CGAs) [8]. The health benefits of CGAs and flavonoids have been recently reviewed [9][10].

2. Development

These secondary metabolites are the biosynthetic products of metabolic pathways that are responsive to environmental stressors or signal molecules associated with adaptation to changing environmental conditions that can affect the phytochemical composition [11]. In response to stress, levels of secondary metabolite phenolics are generally increased [12]. These phenolics are biosynthesized by the pentose phosphate-, shikimate- and the phenylpropanoid pathways and comprise of a common C6-C3 carbon skeleton with a hydroxylated benzene ring. Subclasses include simple phenols, which consist of coumarins and phenolic acids (HCAs and hydroxybenzoic acids) and polyphenols, which consist of flavonoids and tannins [13][14][15][16]. Several of these secondary stress-related metabolites act as antioxidants [17] and are associated with anti-microbial defenses or act as chemical deterrents to inhibit attack by insects and grazing animals [13][18]. For example, CGAs serve as precursors for wound-induced polyphenolic barriers such as lignin and suberin [19]. Isoflavonols and flavonoids are also induced as anti-microbials and protectants against UV radiation [12][20][21].

3. Methyl jasmonate (MeJA) and methyl salicylate (MeSA)

Methyl jasmonate (MeJA) and methyl salicylate (MeSA) are signal molecules of plant innate immunity and plant defense networks that have an effect on the physiological and biochemical processes that may trigger or enhance the production of various secondary metabolites [22]. MeSA is a derivative of salicylic acid (SA) produced through the action of methyltransferases on SA (a key compound derived from the shikimic acid pathway) and is a significant constituent in defense signaling cascades related to systemic acquired resistance (SAR) [23]. Similarly, MeJA is a volatile phytohormone (derived from jasmonic acid (JA) via the octadecanoid pathway), which is also a key mediator in the induced systemic resistance (ISR) response of plants [24]. The JA and SA defense signaling cross-communicate to fine tune defense responses. The former is induced to mediate defense against herbivores and necrotrophic pathogens whilst the SA

pathway is induced in response to biotrophic pathogens [25][26]. These pathways are usually mutually antagonistic as each induce a different set of response genes to a particular pathogen [27]. The reciprocal antagonism of these pathways has been extensively described in *Arabidopsis thaliana*, where SA results in downregulation of JA-responsive genes and similarly a SA-mediated suppression of genes encoding key enzymes in JA biosynthesis has been described[26]. However, JA and SA pathways have also been described to act synergistically in *Nicotiana tabacum* when both hormones were applied at low (10–100 µM) concentrations, and enhancement of gene expression of genes associated JA and SA signaling was observed [28].

Exogenous treatment of plants with phytohormones is conceptually similar to triggering the reponses to abiotic or biotic stressors, as they induce defense-related metabolic pathways, thereby altering the metabolic profiles related to secondary metabolism [29]. Although MeJA and MeSA have been shown to induce the production of secondary metabolites such as phenolics in other plants [30], the effects of these signaling compounds have not been investigated in *B. pilosa.* Here, we report on a metabolomic investigation performed on methanolic extracts from leaf tissues of *B. pilosa* that were treated with MeJA and MeSA and harvested at time intervals of 12 h and 24 h. The results indicate alterations of the metabolome of *B. pilosa* in response to treatment with these phytohomones. Additionally, the profiling of metabolites in *B. pilosa* leaf tissues under exogenous treatment provides an understanding of the interaction between SA- and JA-regulated networks on the altered metabolomes as effected by MeJA and MeSA.

4. Conclusions

MeJA and MeSA are important endogenous signal molecules, which induce and modulate stress responses in plants. Exogenous application of these signal molecules has been shown to alter the metabolome of plants by increasing production of secondary metabolites, often in a defense-related context. In the current study, MeJA and MeSA were used as elicitors with the aim to evaluate possible enhancement of the biosynthesis of health-beneficial secondary metabolites in *B. pilosa.* Metabolomic profiling of the extracted phytochemicals from the leaves treated with MeJA and MeSA indicated differential responses as reflected by perturbations to the metabolomes. Both treatments were shown to enhance levels of some HCA conjugates to tartaric acid (caftaric- and chicoric acids) and quinic acid (mono- and di-caffeoylquinic acids). Of special interest is the annotation of two isomers of 3,5-di-CQA, two isomers of 5-CQA, four isomers of caftaric acid, and two isomers of chicoric acid. This might be a reflection of *de novo* synthesis, but also the molecular dynamics and perturbed equilibria between the isomers due to the stress response. As seen from the annotated metabolites, most of the isomers were identified as possible *cis* isomers, suggesting a possible role of geometrical isomers as biologically active molecules rather than being mere structural artefacts of their *trans*-isomers as previously thought. In addition, increased levels of the annotated flavonoids, quercetin, and kaempferol were observed in the treated plants.

The observed alterations in the phenolic compound profile in *B. pilosa* leaves can be explained through the actions of MeSA/SA and MeJA/JA, known inducers of gene transcription of PAL and cinnamate 4-hydroxylase, leading into the phenylpropanoid pathway. Since MeJA and MeSA both affected similar metabolites originating from the phenylpropanoid pathway, this might be an indication of an inter-connected response in *B. pilosa* to these phytohormones. Time-dependent differences were also observed in the metabolite levels, signifying the transient nature of the induced responses and suggesting stringent endogenous control systems that return the levels of the metabolites associated with the perturbed metabolomes to homeostatic conditions.

As observed in this study, the metabolome of *B. pilosa* is affected by exogenous treatment with signal molecules used as elicitors. These results add novel insights on the metabolomic responses induced by external stimuli on tissues of *B. pilosa.* This highlights the possibility of using MeJA and MeSA (or inducing agents and stimuli acting through these signal molecules) to elicit the production of important bioactive secondary metabolites, such as chlorogenic acids and flavonoids. Future studies based on these results can allow for optimization of elicitor concentrations, elicitation time, and harvest time, to permit the accumulation of the secondary metabolites.

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