Journal of Proteomics 176 (2018) 56–70

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Proteomic analysis of the promotive effect of plant-derived smoke on plant growth of chickpea

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ARTICLE INFO

Keywords:
Proteomics
Chickpea
Plant-derived smoke

ABSTRACT

Plant-derived smoke plays a key role in seed germination and plant growth. To investigate the effect of plant-derived smoke on chickpea, a gel-free/label-free proteomic technique was used. Germination percentage, root/shoot length, and fresh biomass were increased in chickpea treated with 2000 ppm plant-derived smoke within 6 days. On treatment with 2000 ppm plant-derived smoke for 6 days, the abundance of 90 proteins including glycolysis-related proteins significantly changed in chickpea root. Proteins related to signaling and transport were increased; however, protein metabolism, cell, and cell wall were decreased. The sucrose synthase for starch degradation was increased and total soluble sugar was induced. The proteins for nitrate pathway were increased and nitrate content was improved. On the other hand, although secondary metabolism related proteins were decreased, flavonoid contents were increased. Based on proteomic and immuno-blot analyses, proteins related to redox homeostasis were decreased and increased in root and shoot, respectively. Furthermore, fructose-bisphosphate aldolase was increased; while, phosphotransferase and phosphoglycero mutase were decreased in glycolysis. In addition, phosphoglyceraldehyde-3-phosphate dehydrogenase and glutamine synthetase related genes were up-regulated. These results suggest that plant-derived smoke improves early stage of growth in chickpea with the balance of many cascades such as glycolysis, redox homeostasis, and secondary metabolism. *Biological significance:* The current study examined the effects of plant-derived smoke on root of chickpea seedlings using a gel-free/label-free proteomic technique. Based on functional categorization of results from proteomics, proteins related to glycolysis, signaling, transport, protein metabolism, cell wall, and cell were predominantly changed in chickpea. The proteins related to carbohydrate and nitrate pathways were increased, while, those of secondary metabolism were decreased. Physiological analysis indicated that flavonoid, total soluble sugar, and nitrate content were increased in root of chickpea treated with plant-derived smoke for 6 days. Moreover, accumulated protein abundance of glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphosphate aldolase was in agreement with immuno-blot results, which suggests that glycolysis process might be enhanced in root of chickpea in response to plant-derived smoke.

1. Introduction

Chickpea is one of the most important crops grown in world for human and animal consumption [1], because it provides substantial amount of protein. Chickpea has two main types distinguished by seed size, shape, and color; one of them, the seeds of desi chickpea are small and dark color with a large production [2]. Chickpea is rich source of protein and carbohydrates; in addition, it supplies some minerals and vitamins, as well as fatty acids such as oleic and linolenic acid [3]. Chickpea also contains a wide range of phenolic compounds, including flavonols, flavone glycosides, oligomeric, and polymeric proanthocyanidins [4]. To meet the nutritional requirement of the growing population, improvement in the quality of chickpea is utmost important.

Plant-derived smoke generated from fires played an important role in growth and distribution of vegetation [5]. It can re-establish plant communities in semi-arid regions such as chaparral in southern

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; ROS, reactive oxygen species

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https://doi.org/10.1016/j.jprot.2018.01.011
Received 16 October 2017; Received in revised form 10 January 2018; Accepted 24 January 2018
Available online 31 January 2018
1874-3919/

California [6], fynbos in South Africa [7], and kwongan in Australia [8]. Smoke is widely recognized as a germination cue for fire-dependent plant such as *Audouinia capitata* [7] as well as non-fire-dependent plant species like lettuce and red rice [9]. Smoke treatments promote the propagation of economically important plants, because germination percentage is significantly increased [10]. Seeds may be treated with smoke before sowing or immediately after sowing [10]. The isolation of a new compound from plant-derived smoke that stimulates seed germination of many plants assists in unraveling certain processes of post-fire ecology [11]. The compound, which was characterized as the butenolide, was isolated from plant-derived smoke [12] and burning cellulose [13]; furthermore, it was produced by heating combinations of carbohydrates and amino acids [9]. Although plant-derived smoke has a positive effect on plant growth, its biochemical and biological mechanism is not clear.

Regarding plant-life cycle, plant-derived smoke enhanced seed germination of celery/maize [14,15], released seed dormancy in red rice [16], and stimulated root growth of tomato [17]. During the post-germination, the shoot length and biomass of tomato, okra, and bean were increased by plant-derived smoke [18]. Plant-derived smoke improved the vigor of rice [19] and increased the growth of onion plants/bulbs [20]. In addition to seed germination and seedling vigor, plant-derived smoke induced flowering in fire-lily [21], initiated root in *Vigna radiata* hypocotyl cuttings [22], and promoted somatic embryogenesis in geranium [23]. These findings indicate that plant-derived smoke affects different parameters of plants; however, there is no report on the chickpea, which is one of the important crops.

The roles of plant-derived smoke in regulating plant physiology include the following effects: it increased photosynthetic pigments such as chlorophyll *a/b* and total carotenoid; and stimulated the highest total flavonoid content in the leaf of micropropagated banana [24]. Smoke water significantly increased secondary metabolites levels such as flavonoids and phenolics in *Aloe arborescens* seedlings [25]. Application of smoke solution increased the level of K/Ca ion and reduced the level of Na ion in maize; furthermore, it increased total nitrogen, total soluble protein, and antioxidant activities [26]. These findings demonstrate that various metabolic pathways are affected by plant-derived smoke, suggesting that mechanism of developing stage is required to improve the quality of crop.

Although the effects of plant-derived smoke have been evaluated on the germination and growth of various plants, its effect on chickpea growth has not been analyzed so far. In the present study, morphological analysis was performed. Based on morphological analysis, proteomic analysis was carried out. Furthermore, subsequent bioinformatic analysis based on data from proteomic research was performed. To confirm the proteomic results, physiological, immuno-blot, and gene expression analyses were performed.

2. Materials and methods

2.1. Preparation of plant-derived smoke solution

Aerial parts of *Cymbopogon jwarncusa* L. were collected from Kohat University of Science and Technology, Pakistan. Plants were washed with distilled water in order to remove the dust particles and were shade dried. Aqueous smoke solution of plant was prepared as described by Tieu et al. [27]. A portion (333 g) of semi-dried plant was smoldered in a furnace that was air tight and smoke was bubbled through 1 L of distilled water in a beaker to gain concentrated smoke solution, which was filtered through sterilized filter paper and stored at 4 °C.

2.2. Plant material and treatment

Seeds of chickpea (*Cicer arietinum* L.) were sterilized with 3% sodium hypochlorite solution, twice rinsed in water, and sown in 450 mL silica sands in a seedling case (150 mm × 60 mm × 100 mm). The seeds were incubated in a growth chamber illuminated with white fluorescent light (160 μmol $m^{-2} s^{-1}$, 16 h light period/day) at 25 °C and 60% humidity. A total of 15 seeds were sown with appropriate distance in each seedling case. Seeds were supplied without or with 1000, 2000, and 4000 ppm plant-derived smoke for 2, 4, 6, and 8 days for morphological analysis and for 6 days for immuno-blot analysis. For physiological and proteomic analyses, seedlings were treated without or with 2000 ppm for 6 days. Untreated seedlings supplied with water served as controls. Four or three independent experiments were performed for all parameters as biological replicates. The plants used for the biological replicates were sown on different days.

2.3. Protein extraction

A portion (300 mg) of roots was cut into small pieces and placed in the filter cartridge. It was ground with plastic rod for 60 times and 50 μL of cold detergent free lysis buffer containing 7 M urea, 2 M thiourea, 5% CHAPS, and 2 mM tributylphosphine was added to the filter cartridge and was ground for 30 times. Furthermore, 50 μL of lysis buffer was added and ground for 30 times. The resulting suspension was centrifuged at 15000 × *g* for 2 min and the supernatant was collected as total proteins.

2.4. Protein enrichment, reduction, alkylation, and digestion

Extracted proteins (100 μg) in lysis buffer were adjusted to a final volume of 100 μL. Methanol (400 μL) was added to each sample and mixed before addition of 100 μL of chloroform and 300 μL of water. After mixing and centrifugation at 20000 × *g* for 10 min to achieve phase separation, the upper phase was discarded and 300 μL of methanol was added to the lower phase, and then centrifuged at 20000 × *g* for 10 min. The pellet was collected as the soluble fraction [28].

Proteins were re-suspended in 50 mM NH_4HCO_3, reduced with 50 mM dithiothreitol for 30 min at 56 °C, and alkylated with 50 mM iodoacetamide for 30 min at 37 °C in the dark. Alkylated proteins were digested with trypsin and lysyl endopeptidase (Wako, Osaka, Japan) at a 1:100 enzyme/protein ratio for 16 h at 37 °C. Peptides were acidified with formic acid (pH < 3) and the mixed solution was centrifuged at 20000 × *g* for 10 min. The supernatant was collected as digested peptides and analyzed by nano-liquid chromatography (LC) mass spectrometry (MS)/MS.

2.5. Measurement of protein concentrations

The method of Bradford [29] was used to determine the protein concentration with bovine serum albumin used as the standard. A Direct Detect Spectrometer (Millipore, Billerica, MA, USA) equipped with the Direct Detect software (version 3.0.25.0) was used to determine the peptide concentration.

2.6. Protein identification using nano LC-MS/MS

The peptides were loaded onto the LC system (EASY-nLC 1000; Thermo Fisher Scientific, San Jose, CA, USA) equipped with a trap column (EASY-Column, C18-A1 5 μm, 100 μm ID × 20 mm; Thermo Fisher Scientific) equilibrated with 0.1% formic acid and eluted with a linear acetonitrile gradient (0–50%) in 0.1% formic acid at a flow rate of 200 nL/min. The eluted peptides were loaded and separated on the column (C18 capillary tip column, 75 μm ID × 120 mm; Nikkyo Technos, Tokyo, Japan) with a spray voltage of 1.5 kV (capillary temperature: 200 °C). The peptide ions were detected using MS (nanospray LTQ Orbitrap Elite MS; Thermo Fisher Scientific) in the data-dependent acquisition mode with the installed Xcalibur software (version 2.2; Thermo Fisher Scientific). Full-scan mass spectra were acquired in the MS over 400–1500 *m/z* with resolution of 60,000. A lock mass function