Journal of the Saudi Society of Agricultural Sciences (2015) xxx, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

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FULL LENGTH ARTICLE

Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats

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Received 11 June 2015; revised 14 August 2015; accepted 18 August 2015

KEYWORDS

Solanum nigrum;
Phytoremediation;
Sewage water;
Glycine betaine;
Alkaloids;
Proline

Abstract The present study was conducted to evaluate the pollution phytoremediation capacity and pollution tolerance of *Solanum nigrum* in two habitats varied in their pollution intensity with heavy metals. We investigated the relative contributions of the hyperaccumulator *S. nigrum* in eliminating some pollutants from agricultural soils such as Zn, Pb, Ni and Cd in Tanta and in the sewage water irrigated area at El-Gabal El-Asfar (GA) region. The study also concluded that heavy metals pollution resulted in a significant amendment in the primary and secondary metabolic pathways of the plant. The exposure of *S. nigrum* to heavy metals pollution in GA region resulted in a relative accumulation in soluble sugars, soluble proteins and free amino acids in the plant root, stem and leaves, but their levels in berry were to some extent inferior to those of Tanta region. In addition, the exposure of *S. nigrum* plants to sewage water effluents in GA region improved the accumulation of osmoprotectant and antioxidant molecules such as proline, glycine betaine, flavonoids and phenolic compounds. Moreover, the medicinally active alkaloids have accumulated in response to sewage irrigation in various organs of GA region plants comparable with those of Tanta region.

1. Introduction

Soil pollution by heavy metals has become a severe environmental disaster, causing considerable health problems to humans as well as to ecosystems. Heavy metals in contaminated soils cannot be mineralized or degraded to less toxic forms and accumulate through the food chain. Thus, it requires suitable methods for their elimination (Chen et al., 2014). These heavy metals are released into the environment by a large number of human activities such as electroplating, dyes and pigment manufacturing, wood preservation, leather

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2015.08.001
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Please cite this article in press as: Saad-Allah, K.M., Elhaak, M.A. Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.08.001

tanning industry, manufacture of alloys (Nayak et al., 2015), mining wastewater, solid wastes, soil additives, fertilizers, pesticides and air pollutants (Rhoads et al., 1989).

The phytoremediation could be defined as using plants (including trees and grasses) to remove toxic ions from soil and water to mitigate contaminated areas. Phytoremediation is considered as low-cost technology to remediate the polluted regions (Chen et al., 2014). Hyperaccumulator plants can remediate pollutants through several processes such as adsorption, transport and translocation, hyperaccumulation or transformation and mineralization (Meagher, 2000) without suffering metal toxicity or cell damage. The characteristics that make any species useful for phytoremediation include fast-growth with capability to accumulate large biomass, easier and rapid propagation, abundant root system, high metal accumulation ability, tolerance to harsh local soil condition, and inedibility by livestock (Pandey et al., 2012). About 400 species of natural metal hyperaccumulators belonging to 45 families have been documented in the world. These have an innate capability to absorb metal at levels 100 times greater than average plants (Zhou and Song, 2004). Compared with crops, weed plants demonstrate strong stamina to adverse environmental conditions, and high capacity to absorb water and fertilizers (Wei and Zhou, 2006).

Solanum nigrum (black nightshade) is a relatively fast-growing annual medicinal plant belonging to family Solanaceae (Jain et al., 2011) with an erect angular stem, normal tap root, and ovate leaves with dentate margin. The fruits are dull black and globose in extra-axillary umbel inflorescences (Chauhan et al., 2012). *S. nigrum* was found to be a high-biomass hyperaccumulator (Wei et al., 2006) compared with some well-known hyperaccumulators, and it could withstand high heavy metal concentration in contaminated soils (Luo et al., 2011). It is tolerant to adverse environment, so it could fill the gap of known hyperaccumulating plants (Zhou and Song, 2004). For the preceding reason, we decided studying and contrasting the potential of root to shoot then to berry translocation and the superior ability of *S. nigrum* to detoxify and sequester heavy metals in various organs to insure its possible use in phytoremediation and cleaning up of heavily polluted soils paving the way for the cultivation of such areas. Another possibility for our research targets is to explore the disparity in the medicinal components such as alkaloids, flavonoids and phenolics in sites which are polycontaminated with several metals rather than only one metal.

So, the objectives of the present study were to investigate the hyperaccumulation capacity, distribution, bioaccumulation and biotranslocation of various pollutants in *S. nigrum* organs, including the berry, in two habitats differing in their pollution intensity and contrasting the metabolic response of the plant in the two habitats.

2. Materials and methods

2.1. Sampling

S. nigrum plants were harvested during the summer season of 2014 at the maturity stage (before berry turning black) from two different localities in Egypt. The first one was El-Gabal El-Asfar farm at the northern-east of the Egyptian Delta (30°13′N, 31°23′E) and subjected to sewage effluent irrigation for more than 60 years. However, the second one was Tanta city at the middle of Egyptian Delta (30°47′N, 31°00′E), which is an agricultural field irrigated with water supplied from El-Qassed canal, which is a mixture of Nile water and sewage effluent. In addition to the plant samples, soil samples were collected from the rhizosphere of the growing plants.

The harvested plants were separated into root, stem, leaf and berries and washed with tap water several times, and after that with deionized water. The samples were dried at 60 °C in an air-forced oven until dry to constant weight. The dried plant samples were grounded to powders using an electrical mixer and passed through a 2 mm sieve and stored in paper bags at 4 °C for further analysis. However, soil samples were air-dried, grounded and passed through a 2 mm sieve, and then stored in paper bags at room temperature for further analysis.

2.2. Mineral analysis of plant and soil samples

Plant and soil samples were digested with a mixture of 69% HNO_3 and 30% H_2O_2 (5:2 v/v). The concentrations of heavy metals in digested solutions were determined using inductively coupled plasma-optical emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA) at the Central Lab of Tanta University. The used wavelengths for Cd, Zn, Ni, Pb, Fe, Co, As, Cr and Al were 214.4, 206.2, 231.6, 220.3, 283.2, 238.9, 197.2, 267.7 and 394.4 nm, respectively.

2.3. Phytochemical analysis

2.3.1. Preparation of methanolic extract

The fine powdered plant tissues (5 g for each sample) were extracted with 50 ml of 95% methanol for 12 h at room temperature in an orbital shaker (Panasonic, MIR-S100, Japan). The extracts were filtered through Whatman No. 1 filter paper. The residues were extracted twice again as previously and extracts were combined. The combined extracts were concentrated under reduced pressure at 40 °C using rotary evaporator (Heidolph) and adjusted to 50 ml by methanol. Extracts were stored in glass vials at 4 °C until the time of analysis. All the spectrophotometric measurements were carried out using JENWAY 6315 UV/Visible Spectrophotometer (Japan).

2.3.2. Determination of soluble sugars

The total soluble sugar content in the methanolic extract of different plant parts was measured according to the phenol–sulfuric acid method of Dubios et al. (1956) using glucose as a standard and expressed as mg/g d.wt.

2.3.3. Determination of soluble proteins

Total soluble protein content of the extracts was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. The extract or the standard protein was mixed with Coomassie brilliant blue G250 reagent, the absorbance was measured at 595 nm and the results were expressed as mg/g d.wt.

2.3.4. Determination of free amino acids

Amino acid content was analyzed by ninhydrin assays in the methanolic extract using glycine as a standard amino acid according to Lee and Takahashi (1966). Samples or standard

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