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Multi-elemental profiling and chemo-metric validation revealed nutritional qualities of *Zingiber officinale*

Pankaj Pandotra [a], Bhavana Viz [b], Gandhi Ram [a], Ajai Prakash Gupta [b,*], Suphla Gupta [a,c,**]

[a] Plant Biotechnology Department, Indian Institute of Integrative Medicine, Jammu 180001, Jammu & Kashmir, India
[b] Quality Control & Quality Assurance, Indian Institute of Integrative Medicine, Jammu 180001, Jammu & Kashmir, India
[c] Academy of Scientific & Innovative Research (AcSIR), CSIR, Anusandhan Bhawan, 2 Rafi Marg, New Delhi 110001, India

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ABSTRACT

Ginger rhizome is a valued food, spice and an important ingredient of traditional systems of medicine of India, China and Japan. An Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) based multi-elemental profiling was performed to assess the quantitative complement of elements, nutritional quality and toxicity of 46 ginger germplasms, collected from the north western Himalayan India. The abundance of eighteen elements quantified in the acid digested rhizomes was observed to be K > Mg > Fe > Ca > Na > Mn > Zn > Ba > Cu > Cr > Ni > Pb > Co > Se > As > Be > Cd. Toxic element, Hg was not detected in any of the investigated samples. Chemometric analyses showed positive correlation among most of the elements. No negative correlation was observed in any of the metals under investigation. UPGMA based clustering analysis of the quantitative data grouped all the 46 samples into three major clusters, displaying 88% similarity in their metal composition, while eighteen metals investigated grouped into two major clusters. Quantitatively, all the elements analyzed were below the permissible limits laid down by World Health Organization. The results were further validated by cluster analysis (CA) and principal component analysis (PCA) to understand the ionome of the ginger rhizome. The study suggested raw ginger to be a good source of beneficial elements/minerals like Mg, Ca, Mn, Fe, Cu and Zn and will provide platform for understanding the functional and physiological status of ginger rhizome.

1. Introduction

Metals are non-uniformly distributed in the soil and environment. Several factors like industrialization, traffic density and indiscriminate use of chemical fertilizers, pesticides and eco-geological conditions play a deciding role in their quality and quantity. These elements can be accumulated/absorbed by plants through air, water and soil. Literature cites several reports advocating tendency of plants to absorb and accumulate heavy metals in their tissues (Yusuf et al., 2002). Based on the requirement, elements/nutrient ions are categorized into two types: (i) non essential/toxic metals (Pb, Cd, Hg, As) and essential/beneficial metals which includes microelements like Cu, Zn, Mn, Fe, Ni, Se, B, Mo, Na Co Al and macro elements like N, P,S, Ca, K, Mg (Abdul Wahab et al., 2008; Salt et al., 2008). Among the 92 elements identified, plants generally take in 60 elements, out of which seventeen elements are essential for the development and growth of almost all the plants (Satismurti et al. 2013). They play a vital role as structural and functional components of metallo-proteins and enzymes in the living cell in lower doses (Maiga et al., 2005). Recent review by Satismurti et al. (2013) has elaborated the role of ionome in multitude of biological processes including electrophysiology (FRD3), signaling (Ca), enzymology (Cu, Fe), osmoregulation (AKT1) and transportation (RAN1). Plant has an in-built mechanism for ion homeostasis for regulating the optimum concentration and studies have shown that any deviation from the optimum ion concentration affects the normal metabolism, thereby disturbing the ionome of the plant (Baxter, 2008; Salt et al., 2008). Regulation of mineral ion and trace element composition is affected by several factors including soil composition, plant stage and physiology (Baxter, 2009). Studies on multi-elemental fingerprinting have opened up new areas of research and several papers on plant ionomics have been published describing the total ionome profile and their regulation for balanced metabolic processes (Satismurti et al., 2013) and the effect of change in the soil chemical environment on the elemental composition and physiology of the plant (Mihaylova et al., 2013). Studies have shown that leaf ionome can be used for defining the physiological status of the plant based on

* Corresponding author.
** Corresponding author at: Plant Biotechnology Department, Indian Institute of Integrative Medicine, Jammu 180001, Jammu & Kashmir, India.
Fax: +91 191 2569333. IIIM Manuscript No. IIIM/1484/2012
E-mail addresses: ajai747@yahoo.co.in (A.P. Gupta), suphlabg@gmail.com (S. Gupta).

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multivariable metal signature (Baxter et al., 2008). Ionomics is a dynamic network of elements that controls the biochemistry and physiology of the plant and promises to yield new and significant biological insight into nutragenomics and gene/gene networks involved in its regulation (Salt et al., 2008).

The underground stem of ginger (*Zingiber officinale*), rhizome, is a widely used spice, flavorant and an ingredient in the oriental system of medicine. It is listed among the top 20 selling herbs in US. India is the leading supplier of ginger and ginger derived products (oil & oleoresin) to the world market. Literature survey revealed several reports of qualitative and quantitative determination of metals in ginger but, no study pertaining to ginger ionomics has been published. Few papers published on pesticides (Mishra et al., 2007), toxic metal (Gupta et al., 2010; Krejpcio et al., 2007; Naithani and Kakkar, 2005) and beneficial elements (Meena et al., 2010; Otunola et al., 2010; Parveen et al., 2003) have quantitatively analyzed ginger using Atomic Absorption Spectroscopy, Particle induced X-ray emission (Rihawy et al., 2010) and ICPMS in rhizome (Kara, 2009; Tokalioglu, 2012; Nandi et al., 2013). These reports showed wide variation in spectrum of elements in ginger rhizome collected from all across the world. The presence of an element is a complex process controlled by network of gene products critical for uptake, binding, transportation, and sequestration, besides the variation due to environment and the genotype. Therefore, to understand their uptake and regulation, it is necessary to measure maximum possible spectrum of elements contained in a cell, tissue, or organism (Baxter, 2009), advocating establishing its ionome. This will help in proper identification of the best raw material for its use as food, medicine or spice and also for understanding the underlying mechanism of regulation.

The present study involves simultaneous measurement of multi-elemental composition of ginger rhizome using the ICPMS technique and its chemometric validation. Eighteen elements comprising eleven essential, three non-essential and four toxic heavy metals, namely, Zinc (Zn), Manganese (Mn), Magnesium (Mg), Copper (Cu) Calcium (Ca), Beryllium (Be), Iron (Fe), Cobalt (Co), Selenium (Se), Barium (Ba), Potassium (K), Sodium (Na), Chromium (Cr), Nickel (Ni), Arsenic (As), Mercury (Hg), Lead (Pb) and Cadmium (Cd) were analyzed in 46 ginger rhizomes collected from North-western Himalayan ginger growing states of India and their distribution per se in the rhizome. Further, the data was analyzed chemometrically through Cluster analysis (CA), Pearson correlation coefficient (r), and Principal Component Analysis (PCA) to assess and validate mineral nutrient and trace element composition in the ginger rhizome. The study will help in understanding the physiology and regulation processes of the elements in the plant.

2. Experimental

2.1. Collection and preparation of samples

The ginger rhizome was collected from the cultivation fields of farmers from various locations of ginger growing states of Uttarakhand and Himachal Pradesh of North Western Himalayan India (Table 1). The collection was done in the months of April (last week) and May (first week) and the samples were properly labeled. The rhizomes were rinsed under tap water, followed by Milli-Q water thrice (Millipore, Milford, MA) and subsequently, dried at 70°C for 48 h. The dried rhizomes were ground into fine powder, passed through a 0.5 mm diameter sieve, stored in air-tight containers in the dark at room temperature, until digestion.

2.1.1. Chemicals

Milli-Q deionized water (Millipore, Milford, MA) was used throughout the experiment. All the solvents and reagents were of analytical reagent grade (Merck). Prior to use, all the glasswares were soaked in 10% HNO_3 for 12 h, and rinsed with deionized water.

2.1.2. Sample digestion

The homogenized powder (25 mg) of each sample (in triplicates) was weighed in a digestion tube. A mixture of 300 μl of H_2O_2 (30%) and 500 μl of HNO_3 (65%) was added to each sample and digested in a microwave oven following the procedure published by Hansen et al. (2009) at 210°C for 60 min. Subsequently, the cooled digested samples were diluted to a final conc. by 7% HNO_3 and analyzed.

2.1.3. Preparation of standard solutions

Standard solution of each element was prepared by dilutions of 1000 mg/L stock solution (Merck, Darmstadt, Germany) prior to use. Indium solution (1 μg/ml) was used as internal standard for analysis. The quantitative analysis of elements (Pb, Cd, As, Cr, Cu, Fe, Zn, and Ni) in the samples was done using calibration curves obtained from serially diluted stock solution. The solutions were individually diluted with 0.05% HNO_3 and 0.05% HCl to get desired working concentrations. Wide range of linearity is the advantage of ICP-MS, which can be varied from blank value up to hundred μg/ml for each analyte. All the metals were quantified on the dry weight basis. The mean of each sample was calculated by taking the average readings of triplicate sets. All the values are mentioned in mg/kg.

2.2. Analytical procedure

An Agilent 7500a ICP-MS (Agilent Technologies, Palo Alto, CA) was used in this study. Pulse to analog factor (P/A) was determined on the day of analysis, and the tuning of the instrument was carried out using Agilent ICP-MS tuning solution 10 μg/L (Li, Co, Y, Ce and Tl). The ICP-MS operating parameters for ginger sample analysis were the following: plasma power 1550 W; RF matching 1.66 V; sample depth 9 mm; torch-H 0.4 mm; torch-V 0.1 mm; carrier gas 0.93 L/min; makeup gas 0.19 L/min; Aux gas 0.95 L/min; plasma gas 15 L/min; nebulizer pump 0.1 rps; sample load 8 ml; nebulizer glass concentric; sample tube (mm, o.d.) 1.6; internal standard tube (mm, i.d.) 0.5; rinse time 20 s/standard spray chamber temp 2 °C; and interface cones Ni. Total analysis time was 7 min. Data acquisition was done in spectrum analysis and full quant mode. Agilent 7500a ICP-MS interfaced to an IBM personal computer system (controlled by chemstation software) was used for the analysis.

2.3. Statistical analysis

The descriptive statistics of all the 18 elements in ginger rhizome was performed in Window 2007 Excel worksheet. For Cluster Analysis (CA) rectangular similarity matrix was generated using correlation coefficient. Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) based dendrogram was plotted using NTSYS pc software Ver2. (Rohlf, 1996). Pearson′s correlation coefficient (r) was computed using SPSS software (SPSS, 2003). Two-tailed significant correlation was calculated at probabilities $P < 0.01$ and $P < 0.05$ levels. Same data was validated by Principal Component Analysis (PCA) following Kaiser′s rule and Quartimax rotation (XLSTAT 2010.5.03/ SPSS software, 2003).