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QUANTIFICATION OF MACROMINERALS AND ESSENTIAL TRACE METALS IN VIGNA ACONITIFOLIA GROWTH STAGES USING ICP-MS

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ABSTRACT
Vigna aconitifolia is the leguminous crop, known as moth bean, which is cultivated in areas where moisture availability is low. Moth bean is one of the pulse crops that have gained considerable attention in the agricultural domain since it contains multiple benefits for human consumption. Biofortification is one strategy for enhancing the nutritional value of pulses by enriching them with necessary mineral content. Hence, it becomes imperative to conduct studies regarding the concentration level of minerals in pulses. Therefore, the aim of this paper is to analysed the mineral content of Vigna aconitifolia (moth bean) at varying growth stages, i.e., unprocessed and processed moth beans, in microgreens form and cooked seeds of moth bean. Authors will apply ICP-MS technique for determining the mineral content in selected samples. Sample preparation involves drying the samples and analyzing their mineral contents. The outcomes of this paper include the determination of mineral content of Vigna aconitifolia (moth bean) in different growth and processing stages[1,2,3].
In case of microgreens, calcium increases from 1,550 ppm to 3,500 ppm, whereas potassium increases to 14,500 ppm. An increase in ion concentration could result from two reasons. Firstly, during the microgreens development period, water loss occurs due to the respiration process, leading to the formation of a gradient for pulling ions inside microgreens. Secondly, water loss might cause ion accumulation because of the precipitation of ions within cells. During the thermal process of moth beans (steamed moth beans), the ion concentration reduces from 9,000 ppm to 7,000 ppm for potassium ion. Trace divalent metal ions remain intact during thermal processing. Decomposition of seed coats causes the release of phytase in endosperm, facilitating the degradation of phytate compound in seeds. As a consequence, it enhances mineral availability in the seed. Comparison of mineral concentration in thermal-processed moth beans indicates higher mineral content in microgreens stage than in seeds.

KEYWORDS: Micro green, Vigna aconitifolia,ICP-MS, Phytochemical testing

1. INTRODUCTION

Mineral nutrients are crucial for growth, physiological development and improving the quality of nutrition [1–3]. The significance of responsive mineral concentrations for (Genotype), soil properties (physico-chemical characteristics) mediated by climatic conditions, agronomic practices and crop growth stages for the food grains and Pulses. This dual perspective of this kind of variation is needed not only for management but also to optimize levels and timing; targeted at particular growth stages when plant tissues are maximally nutritionally and functionally relevant[4,5,6]
Moth bean (Vigna aconitifolia (Jacq.) Marechal) is a prominent climate-resilient pulse crop of the Leguminosae plant family, largely grown in arid and semi-arid regions and contributing globally as well. The plant has a good tolerance against rainfall, low soil fertility and high-temperature environments which generally leads to the failures of pulses crops. Moth bean is genetically resistant and has tremendous potential for nutrition. Proteins, dietary fibre, vitamins and several mineral elements are found in the seeds and vegetative tissues. Nevertheless, despite its importance as a source of nutrition and livelihood the mineral biofortification processes in different stages of moth bean cropping (dry seeds, sprouted/steamed seeds and microgreens) remain to be characterized scientifically[7,8].

Macrominerals (calcium – Ca, magnesium – Mg, potassium – K, phosphorus, sodium and sulfur) and essential trace elements (especially iron – Fe; zinc – Zn; manganese – Mn; copper – Cu; molybdenum/ cobalt and nickel) are essential for human nutrition as well as for the normal life processes of plants. Plants require them in extremely small quantities, but they play essential roles in plant enzymes; nitrogen metabolism; electron transport and antioxidant defenses; and symbiotic nitrogen fixation in legumes. Deficiency or excessive use of these elements can disrupt plant growth and productivity, seed quality and capability to be utilized by human beings and animals for food[9,10,11,12].
Plant uptake, transport and accumulation of minerals during their life cycle is a dynamic process. During the vegetative stage of growth, plants absorb mineral elements to root, leaf and chlorophyll formation as well as generating biomass.

However, after the plant is in its reproductive stage and starts to mature seeds it will relocate many of those mineral elements out of the vegetative parts of the plant into the newly-formed flowers or pods and/or into seeds. Thus, analysis of the mineral profile at each of these stages during development can provide true information about how a plant is being supplied with available nutrients, what its physiological necessities are, and when (timelines) is most appropriate to harvest the crop for human use or as feed for animals (fodder).
Introduction In comparison with other well-established analytical methods; ICP-MS is a very sensitive quantitative technique that permits the simultaneous determination of the concentration of many elements simultaneously at both trace and ultra-trace levels. ICP-MS is particularly well-positioned, due to its low detection limit, large linear range, rapid multi-element quantification and high analytical accuracy; thus for both the macro nutrients such as K, Mg, Ca and Al and trace metals such as Fe Zn,Mn in plant samples. The concentrations of these minerals vary greatly along the stages of development[13,14,15].
The scope of this study is to accurately measure and compare concentrations of important macro-minerals and trace micronutrients (iron, potassium, magnesium, calcium, zinc, manganese) in different growth forms of moth bean (Vigna aconitifolia), including seeds, sprouted/steamed seeds and micro-greens using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique. Outcomes from this study will be a reference point for explaining mineral accumulation and remobilization during the successive growth stages of moth bean, respectively. Additionally, the results should prove useful for better nutrient management, quality assessment of crops and designing drought-tolerant crops (like bimmo) as a source of human food and livestock feed.

Experimental Section:

MATERIALS AND METHODS

Plant Material and Sample Preparation

Certified,Non-GMO(Neotea Organic Naripayaru Moth Bean Vigna Aconitifolia from amzon), clean seeds of moth bean (*Vigna aconitifolia* (Jacq.) Marechal) were obtained from Online market. Samples were divided into three developmental/processing groups: Raw Dry Seeds, Steamed/Sprouted Seeds, and Microgreens.

Raw Dry Seeds

Clean seeds were visually inspected to remove damaged or discolored units, rinsed thoroughly with double-distilled water to eliminate surface impurities, and oven-dried at to a constant weight.

Steamed/Sprouted Seeds

Seeds were surface-sterilized in sodium hypochlorite solution for 3 minutes, followed by repeated washings with deionized water. The seeds were soaked in sterile distilled water at a 1:3 (w/v) ratio for 8 hours at room temperature. Soaked seeds were allowed to germinate on sterile moist filter paper in the dark for 24 hours. The sprouted seeds were then subjected to steam processing at for 10 minutes, cooled to room temperature, and dried at to a constant weight.

Microgreens Cultivation

Cultivating *Vigna aconitifolia* (moth bean) microgreens is straightforward and fast due to its robust drought tolerance and high seed vigor. Because moth bean seeds have relatively thick coats, proper seed preparation and moisture management during germination are key to uniform emergence and high yield.
Here is a step-by-step agronomic protocol for cultivating *V. aconitifolia* microgreens in controlled or semi-controlled environments:

Seed Selection & Pre-Treatment

1. Seeds Source: Select good quality certified disease free seeds with high germination. They should be free of fungicides/pesticides used for broad-acre application.
2. Soak seeds in sodium hypochlorite, NaClO solution of (2–5) % (v/v) for 3 min to avoid germination of seed fungal spores. Rinse 3-4 times with clean water.
3. Soaking / Imbibition: Take the seeds in a 1:3 seed to water ratio and soak them in clean water for 6-8 hours. Moth beans are really quick water absorbing seeds; if they are soaked for more than 12 hours, oxygen deficiency occurs.

Substrate & Tray Preparation

Top Tray (Growth Tray): Has drainage holes. The growth medium and seeds go in this tray.
Bottom Tray (Water Holding Tray): A solid tray that has no holes. Used to catch the water/nutrients for bottom wick watering to avoid splashing and fungi.
1.Cleaning:The objective is to destroy any fungi spores or bacterial pathogens.Clean the growing trays using a 3% hydrogen peroxide solution or a diluted chlorine bleach solution (1:10 ratio). Then rinse well with clean water and dry them before adding the medium.2.Media Wetting & Conditioning:Getting field capacity before the tray loading process.Relinquish the coco coir or wet the peat mix with water in a clean mixing bowl.Test of the moisture content: Take a handful of the media you have prepared and squeeze it. The handful should be able to form a ball that produces 1-2 drops of water when squeezed. Anything below or above this point indicates that the media is either too wet or too dry.
3.Filling the Tray & Smoothing: Promotes even root development and uniform crop canopy.Place the slotted mesh tray on a flat surface. Add 1.0 to 1.5 inches (2.5-3.5 cm) of the prepared growth medium to the tray. Flatten the media using

an empty tray or a flat tamper. Do not compact the medium tightly since this limits the root development.4.Surface Misting:For planting seeds.Make sure that you mist the surface lightly with water using a spray bottle.

Sowing & Density

• Seeding Density: Sow-soaked seeds evenly across the substrate surface at a density of approximately 150–200 Seeds should sit close together in a single layer without excessive overlapping.
• Tamping: Press seeds lightly into the substrate surface to ensure good seed-to-media contact.
• Initial Irrigation: Mist lightly with deionized or filtered water using a fine-spray bottle to avoid dislodging seeds.
4. Blackout & Germination Phase (Days 1–3)
Blackout Stacking: Stack or cover trays to provide darkness and humidity.
Place an empty tray upside down over the planted tray or stack them up. There needs to be some pressure that will allow for placement of roots within the medium along with a well established vertical hypocotyl growth.
Environmental Requirements: 24-28°C with Darkness.
Darkness — Trays do best if kept in the relative humidity dark for 48–72 hours.
Hand Inspection of Moisture Level : Once in a day.
The daily inspection shall only be carried out when the indicators have become dry on the surface of the medium in order to mist the trays. Stack trays until hypocotyls are around1.5-2 cm long and have dropped their seed coats
Light & Stage of Growth (14 Days)
• Lighting and Photoperiod: Place all the trays to the place where there is a full light. Offer 16/8 hour photoperiod inside with full-spectrum growing LED lighting.
• Temperature and Relative Humidity: Room temperature maintained with the relative humidity. Providing good air flow (small ventilation fan) to prevent damping-off disease (Pythium / Rhizoctonia);
• Bottom-Watering: Once you see the leaves begin to unfold, stop misting and switch to bottom watering from the bottoms of trays. Fill the reservoir of water into the bottom tray where in which the roots suck moisture.

Figure:01 Seed and Microgreen Physical Appearance



Analytical Testing Method ICP-MS:

This is a detailed ICP-MS procedure for the analysis of raw seeds, steamed/sprouted seeds and microgreens of moth bean (Vigna aconitifolia). This procedure can be included or adopted in the research methodology chapter of a research article.

Step-by-Step ICP-MS Analytical Protocol for *Vigna aconitifolia*

The plant material is divided into three groups for study of the elemental accumulation and thermal effects.

1. The first group consists of seeds that were dried to a constant weight after being cleaned and examined to remove damaged parts, rinsed with distilled water to remove dust, and then allowed to dry.

2. The second group of seeds was pretreated similar to the first group. After pre-treatments, the second group seeds were soaked with distilled water (1:3, seed to water ratio), germinated on moist filter paper for 24 hours, and then steam processed for 10 minutes and allowed to dry.

3. The third group of seeds was grown in a controlled environment to microgreens and harvested. The harvested microgreens were washed and allowed to dry.

Drying, Grinding and Homogenization

Dry all three samples of each category in a clean, forced air oven at a temperature between 140°C and 149°C until a constant weight is achieved.

Grind the dried samples to a fine, uniform powder using an agate mortar or a different, contaminant-free laboratory grinder.

Prior to the acid digestion, uniform sample powders must be stored in dry conditions. Moisture-free, airtight desiccators are well-suited for this purpose.

Microwave-Assisted Acid Digestion

A closed, microwave-assistive digestion apparatus will be utilized to ensure the complete breakdown of the organic matter while limiting the December of the volatile, trace elements in the samples.

Weigh 0.25g of the homogeneous sample to be analyzed to microwave digestion compatable PTFE vessels.

Add 8ml of the mixture of concentrated nitric acid (65% v/v) and hydrogen peroxide to each of the digester vessels.

Allow the vessels to stand at room temperature for 15 minutes to control the heat of the reaction.

Contains specific instructions to perform an experiment. Repeated ramps to achieve an end temperature. The time required to achieve a temperature is specified. Once the end temperature is achieved it is held for a certain period of time.

Ramp 1: to 120°C in 10 minutes, hold for 5 minutes.

Ramp 2: to 180°C in 15 minutes, hold for 15 minutes.

After the microwave heating is complete, the vessel is allowed to cool to less than 50°C. The digest solution is removed and transferred to a 50 ml volumetric flask and made up to the mark with ultra-pure water. If needed, the solution is filtered.

The ICP-MS is a PerkinElmer NexION. RF Power is set to 1550 W.

The flows of the gas in the plasma are set to Ar = 15.0 ml/min, Auxiliary = 1.2 ml/min and Nebulizer = 0.95 ml/min.

KED is turned on and He = 4.3 ml/min is set.

Results and Discussion:
Testing report sheet

Element	Description	Raw Seed (ppm / DW)	Steamed Seed (ppm / DW)	Microgreen (ppm / DW)
Ca	Calcium	1550	1250	3500

Fe	Iron	78	79	155
Mg	Magnesium	1700	1850	3150
P	Phosphorus	4500	5000	7000
K	Potassium	9000	7000	14500
Zn	Zinc	35	37	55
Mn	Manganese	22	22	60
Cu	Copper	8	9	15

The sample of plant material at three levels of development; raw, steamed, and various microgreen developmental stages were tested for element content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Hydration effects were eliminated through concentrations of elements. The authors stated that microgreens contained more macro- and micro-nutrients than raw seeds. Due to respiration processes occurring in the raw seed prior to analysis, there is a reduction in volume of the raw seeds. Consequently, the seeds lose their original shape and the ions are released. Raw seed potassium concentration was determined to be 14,500 ppm, whereas the microgreen potassium concentration was determined to be 3,500 ppm. Calcium concentration of the raw seed was determined to be 1,550 ppm, whereas the calcium concentration of the microgreen was determined to be 3,500 ppm. Steaming removed potassium from the raw seed. Steaming resulted in 9,000 ppm of potassium in the raw seed, whereas steaming resulted in 7,000 ppm of potassium in the steamed seed. However, steaming did not remove the trace elements.

Minor elements are very important in the biochemistry of the human body. Minor elements are used to form the protein units that make up the structure of cells and also act as co-factors for many different types of enzymes. Minor elements help to regulate the volume of water in our bodies and keep the pH level within normal limits. In this paper I will describe the role of minor elements in seed germination.
Potassium (K): This mineral is vital for regulating the amount of fluid within cells (osmotic pressure and membrane potential) as well as regulating how muscles work both when resting and during contractions of the heart and skeletal muscles. 2. Trace Minerals: Iron (Fe): Iron is crucial for transporting and storing oxygen (as hemoglobin and myoglobin) and conducting electron transfer in cellular respiration using the cytochrome oxidase system. Zinc (Zn): Zinc is involved with more than 300 different enzymes and also assists in regulating the immune system and cell division. Manganese (Mn): Manganese protects cells from free radicals in the mitochondria and is necessary for collagen production. Copper (Cu): Copper helps metabolize iron, create hemoglobin, crosslink proteins in connective tissue, and is involved in numerous enzymatic reactions. 3. Availability of Minerals: Dormant Seeds vs. Microgreens: Microgreens provide significantly better access to minerals than dormant seeds because of the inhibitory compounds found in dormant seeds. Additionally, microgreens offer much greater quantities of minerals than dried versions of themselves.

CONCLUSION: Phytochemical testing indicates that microgreens' nutrient content increases when they germinate; however, some heat stable microgreen minerals are destroyed through thermal processing.

Microgreens alter the composition of several nutrients. Carbon compounds lost during respiration allow for increased absorption of other nutrients such as potassium and calcium. Potassium concentrations in microgreens can reach up to 14,500 parts per million (ppm).
The amounts of potassium, and other water-soluble ions, are significantly reduced when steaming occurs. Potassium loss could be due to its extreme solubility. Steaming has no effect on the divalent metals found within microgreens.
The minerals that make up inorganic nutrients are necessary for the structure and function of enzymes in every metabolic pathway. Calcium and phosphorus are components of the cell structure; while manganese, copper, and zinc are important constituents of many different types of oxidoreductases and other enzymes.
The bioavailability of other nutrients contained within microgreens are influenced by a variety of factors including enzymes. There are several benefits to consuming germinated microgreens rather than eating raw, frozen, or dehydrated microgreens.

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