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Water Infusion of IR-Bagendit Rice Leaves from Various Locations in Central Java as a Candidate Material to Prevent a Heavy Metal Exposure

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Abstract: The use of heavy metals in many industries provides multiple advantages but can pollute the environment and cause health problems that endanger living beings' lives. Moreover, the clinical use of chelating materials causes discomfort and neurotoxicity. Therapy for heavy metal poisoning can be implemented using chelating materials, such as DMSA, BAL, DPCN, and EDTA. Water infusion of IR-Bagendit rice leaves contains metallothionein proteins, potentially becoming a candidate material to prevent heavy metal exposure. The metallothionein protein can bind and detoxify heavy metals in covalent. IR-Bagendit is plentifully found in Central Java. However, no study has investigated the metallothionein protein content of IR-Bagendit leaves from different locations to prevent heavy metals. This study aimed to determine the metallothionein protein content of IR-Bagendit leaves from Boja, Batang, Blora, and Weleri, Jawa Tengah, Indonesia. The research sample was the rice leaves aged four to six weeks from Boja, Batang, Blora, and Weleri. The study collected five snippets from each region. The collected rice leaves were made in infusion and examined using the ELISA method. This study revealed that the metallothionein protein levels of IR-Bagendit leaves, sequentially from the highest to lowest, are from Blora (380.636 ng/L), Weleri (252.189 ng/L), Batang (121.748 ng/L), and Boja (28.832 ng/L). The study concludes that the IR-Bagendit from Blora has the highest metallothionein protein content and potentially becomes a material candidate to prevent heavy metal exposure.

Keywords: water infusion of IR-Bagendit rice leaves, heavy metals, candidate material.

混合資源和相互作用模式對偏遠地區可再生能源管理的影響

摘要：在許多行業中使用重金屬提供了多種優勢，但會污染環境並導致危害生物生命的健康問題。此外，螯合材料的臨床使用會引起不適和神經毒性。重金屬中毒的治療可以使用螯合材料，如不錯，但是、DPCN 和乙二胺四乙酸。紅外燈 水稻葉片的水浸液含有金屬硫蛋白，有可能成為防止重金屬暴露的候選材料。金屬硫蛋白可以共價結合重金屬並使其解毒。红外线-巴根迪特在中爪哇有很多。然而，沒有研究調查來自不同位置的红外线-巴根迪特葉子的金屬硫蛋白含量以防止重金屬。本研究旨在確定來自印度尼西亞爪哇登加的顏色、莖、布洛拉 和韦莱里的红外线-巴根迪特葉子的金屬硫蛋白含量。研究樣本是來自顏色、莖、布洛拉 和韦莱里的 4 至 6 週齡的稻葉。該研究從每個地區收集了五個片段。收集的稻葉浸液製成，並使用 酶聯免疫吸附試驗方法進行檢查。該研究表明，紅外燈葉片的金屬硫蛋白水平從高到低依次為布洛拉（380.636 納克/升)、韋萊里(252.189 納克/升)、莖（121.748 納克/升）和顏色（28.832 納克/升）。該研究得出結論，來自布洛拉 的红外线-巴根迪特具有最高的金屬硫蛋白蛋白質含量，並有可能成為防止重金屬暴露的候選材料。

关键词：和巴根迪特 稻葉浸水、重金屬、候選材料。

1. Introduction

Heavy metal is a metal type or element that has high

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criteria of density or metal elements and can be used for various industrial purposes [1, 2, 3]. There are two types of heavy metals: essential and non-essential types (4). The crucial heavy metals consist of Zn, Fe, Cu, Mn, Se, and Co. The right amount of essential metal is highly needed by the organism. However, the significant metal can cause toxic effects.

Meanwhile, the non-essential metals consist of Cd, Pb, Sn, Cr, As, and Hg. The non-essential metal is the most toxic or toxic metal that can cause poor or adverse effects on human health [5, 6]. Various industries that utilize heavy metals potentially pollute the environment and harm living creatures, including humans [5, 7, 8].

Several efforts have been developed to treat heavy metal toxicity, and one of them is the use of a chelating agent administered in the early stage of treatment. The chelating materials are 2.3-dimercaptosuccinic acid (DMSA), dimercaptopropanol (BAL), or d-penicillamine (DPCN) [9, 10, 11]. The clinical use of ethylenediaminetetraacetic acid (EDTA) and 2.3-dimercaptopropanol (BAL) is now restricted because the inconvenient parenteral administration results in toxicity and likely increases the neurotoxicity of several metals [12]. Therefore, chelating materials that can reduce or prevent heavy metal exposure are pivotal [13, 14]. One of the chelating materials is metallothionein protein [15, 16]. The metallothionein protein is rich in sulfhydryl groups that can bind heavy metals covalently [17]. Metallothionein is a small-sizedcytosolic protein (25-82 aa, 2.5-8.0 KDa) and cysteine-rich protein functioning to chelate heavy metals with a high affinity in thiol groups of cysteine (Cys) (18-23 Cys contained a conserved region of gene). The existence of metallothionein can be detected using the ELISA method [18].

The MTs gene can be isolated from plants and expressed in various tissues and organs, such as roots, stems, leaves, flowers, fruits, and seeds from variously different plant species [19]. The metallothionein protein content of roots, stems, leaves, seeds, or fruits on rice, corn, soybeans, and chickpeas have been investigated. The investigations discover that rice leaves contain the most metallothionein protein [20]. Different varieties of rice leaves, such as red glutinous rice, black glutinous rice, white glutinous rice, red rice, Chiherank rice, umbuk rice, Ciliwung rice, IR-bagendit rice, and IR-64 rice, have also been observed. The observation discovers that IR-Bagendit leaves have the most metallothionein protein. The results of the isolation, similarity identification, and qualitative expression of the metallothionein gene in different varieties of rice leaves, such as IR-Bagendit, Inpari, IR 34, IR 35, Umbu, Umbuk, and Glutinous, show that the quantification and purity of DNA or RNA are most commonly found in IR-Bagendit rice leaves.

Meanwhile, the silico sequence analysis of the metallothionein genes on rice leaves based on the NCBI data reveals that the metallothionein genes are located in Oryza sativa chromosome 3 functioning to stress-inducible proteins in drought conditions, land conditions, and waters containing metals. Consequently, metallothionein can be used as a biomarker of heavy metal exposure [21]. The results of previous studies have shown that IR-Bagendit rice leaves have the highest metallothionein, and the leaves provenly prevent liver and kidney failure due to Pb exposure [22, 23].

IR-Bagendit rice is widely grown in various regions of Central Java, including Boja, Blora, Batang, and Weleri. However, no study has investigated the metallothionein protein content of the IR-Bagendit rice leaves from various locations to discover if the content from one region is similar to that from another. An investigation on the metallothionein protein content of IR-Bagendit rice leaves from various locations of Central Java is highly necessary to help the improvement of using the leaves as an in vivo chelating material for heavy metals

2. Methods/Materials

This research employed an observation method. The IR-Bagendit rice leaves aged 1 to 1.5 months were collected randomly from Boja, Blora, Kendal, and Weleri. The collected rice leaves were then cleaned and made into an infusion.

2.1. Maps of Research Sampling Sites

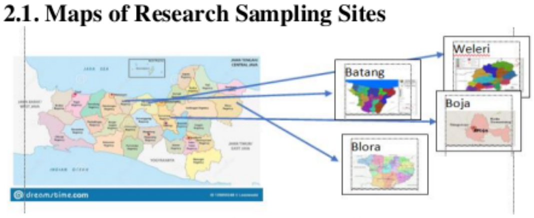

Fig. 1 Maps of research sampling sites

2.2. The Process of Making Infusion

Rice leaves of various varieties had been cleaned and washed with running water before they were chopped into smaller pieces. Then, weigh them as much as 100 g. Next, put the pieces into pan A, add distilled water as much as 1 liter, and close the pan. Then, add water in pan B (as a water bath) sufficiently until the upper part of pan (A) was submerged partially. Heat the pan for 15 minutes until the temperature inside pan A reached $90^{o}C$ while stirring occasionally. The infusion was strained while it was hot using a flannel fabric. The supernatant is infusion.

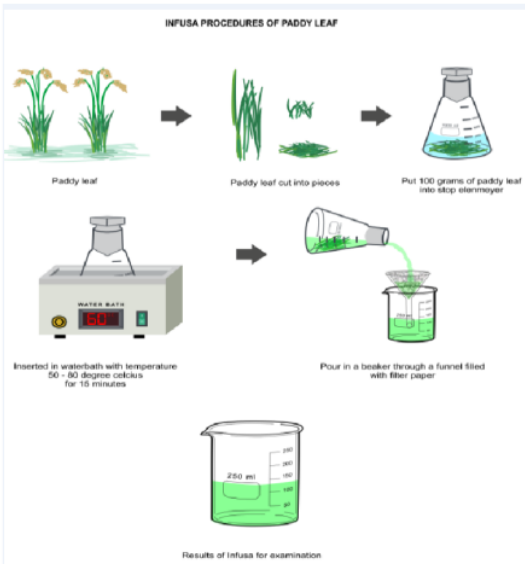

Fig. 2 The process of making an infusion

According to the region, the obtained infusion was labeled, and its protein metallothionein was examined using the Elisa method.

The metallothionein protein on the IR-Bagendit rice leaves was measured using the Elisa method.

2.3. The Principles of Examination

The microplate was coated with specific MT antibodies. Standards and samples were added into the microwell appropriate to the biotin conjugated on the specific polyclonal antibodies for MT. Next, avidin conjugated to horseradish peroxidase was added to each well and incubated. The substrate solution was added to each well. Color changes occurred in wells containing MT, biotin-conjugated antibodies, and avidin-conjugated enzymes. The reaction of the enzyme-substrate was terminated by adding sulfuric acid solution. Meanwhile, the colo 9 changes were measured by spectrophotometry with a wavelength of 450 nm 2 nm. The concentration of MT in the samples was determined by comparing their OD to the standard curve.

2.4. Procedures

All reagents were prepared a room temperature and homogenized. Each well was added 100 standards, blanks, and samples, covered with a sealer, and incubated for two hours at a temperature of 370 C. Then, add 100 ml of Reagent solution detection to each well. Cover with a sealer. The incubation lasted for an hour at 370 C. Aspirate each well properly. Then, wash the well with wash buffer approximately 400 ml using a spray bottle, multi pipette, dispensers, or auto washer. Repeat this step three times. Then, add 100 ml of Detection Reagent Solution B to each well. Cover each well with a new sealer, and incubate them for an hour at 370 C. The laundering step was repeated five times. Add 90 ml of Substrate Solution to each well. Then, cover them with a sealer, and incubate them for 15 to 30 minutes at a temperature of 370 C. Protect from light. Add 50 ml of Stop Solution to each well. If color changes did not appear, press the well to ensure thorough mixing. Read the spectrophotometer with a wavelength of 450 nm. The levels of metallothionein protein were obtained by measuring the sample absorbance in the spectrophotometer. Then, the levels were put in the experimental curve formula obtained from the standard solution.

3. Results and Discussion

3.1. The Metallothionein Levels from Different Varieties of Rice Leave

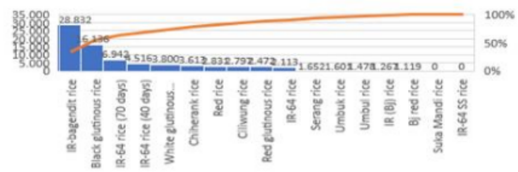

Fig. 3 The Description of metallothionein (MT) levels of different varieties of rice leaf

Figure 1 shows that 17 varieties of rice leaves have been investigated. They are IR-bagendit, Black glutinous rice, IR-64 rice (70 days), IR-64 rice (40 days), White glutinous, Chiherank rice, Red rice, Ciliwung rice, Red glutinous, Serang rice, Umbuk rice, Pennant rice, IR (Bj) rice, Bj red rice, Like a shower of rice, and IR-64 SS rice. The highest levels of metallothionein were found in the IR-Bagendit rice leaves (28.832 ng/L).

The IR-Bagendit rice leaves were collected from several locations in Central Java, namely Boja, Batang, Blora, and Weleri, to examine the metallothionein levels. The result of this investigation is presented in Table 1.

Table 1 The levels of metallothionein (MT) in IR-Bagendit rice leaves from various locations

Locations	The Levels of MT	
	n	Means (ng/L)
Boja	5	28.832
Batang	5	121.748
Blora	5	380.636
Weleri	5	252.189

Table 1 proves that IR begendit from Boja, Batang, Blora, and Weleri has different metallothionein levels. The highest level is from Blora (380.636 ng/L), while the lowest level is from Boja (28.832 ng/L).

3.2. Discussion

Although IR-Bagendit has the highest metallothionein levels, the varieties of IR-Bagendit leaves investigated show diverse metallothionein levels. This study investigated IR-Bagendit rice leaves from several regions of Central Java, namely Boja, Batang, Blora, and Weleri. The investigation discovered that IR-Bagendit rice leaves from Blora had the highest metallothionein level. The metallothionein levels in IR-Bagendit rice leaves can be used to prevent heavy metal exposure.

Molecularly, the metallothionein gene notation of rice from locus sequence typing locus_tag=" OSJNBa0003G23.9" is similar to the stress-of inducible protein STI GB: CAA56165 GI:872116 [Glycine max]. codon_start=1. Product= "putative stress-inducible protein.

protein_id="AAK00971.1.translation="MKKCLEV LIPVTFRKELTYHIWNSA VTYTPVVTTAIQLDPTDATLHSNRSFCYLKSGEA REALVDAKTCIGLKP WPKGYYRKGAALMSLKEYKEACDAFMDGVKL DPASGEMHEAFWEAAAALKKKHLAGKTVSSFD"

The metallothionein genes are located on chromosome 3, functioning as a protein induced by environmental stresses, such as the presence of metallic contaminants [21]. The expression of the gene encoding metallothionein increases the induction of heavy metals, such as Cu, Zn, Ni, and Cd; metallothionein (MTs) is a well-defined ligand binder of heavy metals on plants [24]. Using metallothionein as a material candidate to prevent heavy metal exposure can be considered. The results of this research showed that the affinity of metal ions for the recombinant form of ZmMT1 could be set as follows: Cd (II)> Pb (II)> Zn (II). The observation showed that the chelator agents, such as ethylenediaminetetraacetic acid (EDTA) and ATP, accelerated the oxidation of ZmMT1 with the following order: EDTA ≫ L-histidine> ATP ≈ citric acid. Meanwhile, the buffer commonly used to improve the reactivity of the ZmMT1 with DTNB was set in the following order: PBS> Tris-HCl> HEPES [25].

5. Conclusion

This study concludes that the highest metallothionein protein was found in IR-Bagendit leaves from Blora, Central Java. The IR-Bagendit leaves are a potential candidate material to prevent heavy metal exposure.

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