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Research Article
Root Inoculation with *Pseudomonas putida* IFO 14796 for Improving Iron Contents in Maize Grain

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Abstract
Background and Objective: Anemia that caused by intake the low iron staple food is a global issue. Biofortification through PGP (plant growth promotion) intervention was a new strategy to improve mineral content in staple food. This study aimed to improve iron content in maize grain through root inoculating with *Pseudomonas putida* IFO 14796. **Materials and Methods:** Experiment was carried out by randomized group design. Roots plant was inoculated *P. putida* IFO 14796 (5.18×10^{7} C.F.U g^{-1}) used as treatment and uninoculated as a control. The stem size mean of corn plant was measured after 55 days in cultivated. The iron contents in maize grain after 30 DAP (days after pollination) were analyzed using the Atomic Absorbance Spectrophotometer (AAS) method. **Results:** The stem size mean of the corn plant with the treatment of *P. putida* IFO 14796 was 65.09 cm and control 52.39. The stem size mean of corn plant was significantly different at $p<0.05$. The higher iron content in maize grain 10.1117 mg kg^{-1} was obtained from root inoculated *P. putida* IFO 14796, while uninoculated was 8.5130 mg kg^{-1}. Improving iron content in maize grain up to 18.79% after 30 DAP (days after pollination). **Conclusion:** It is concluded that Iron content in maize grain can be improved through root inoculating with *P. putida* IFO 14796.

Key words: Iron staple food, maize grain, pseudomonas putida, biofortification, anemia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malnutrition and chronic diseases are global problems, especially in developing countries. In Indonesia, the prevalence of anemia remained as a moderate category of public health problem (20-39.9%), especially in children and pregnant women[1]. Low nutrient intake seems to be a significant determinant factor as a cause of iron deficiency[2]. Improving the iron status of the community can be carried out through supplementation, fortification and biofortification. Biofortification of staple food has been done using transgenic, plant breeding and agronomic intervention[3]. It has been done on various food products, such as rice, corn[4,5], soybeans (*Glycine max*)[6], tomato[7], carrots[8] and turnips (*Raphanus sativus*)[9]. The iron biofortification with agronomic interventions in staple foods, such as rice, wheat and corn was believed to be a sustainable strategy to overcome iron deficient in humans[10]. Agronomic intervention using *P. putida* was one of the important strategies to improve the iron contents in maize grain seed because it was one of the most dominant species found in root tissue of corn plant as endophytic[11]. In addition, *P. putida* can produce Indole-3-Acetic Acid (IAA) and siderophores plays an important role in stimulating the growth of roots and shoots and plant weight, number of leaves, number of seeds, iron contents of corn seed and protecting plants against the phytotoxin[12,13] and it can be reduced[14,15] Fe^{3+} to $Fe^{(2+)}$.

A wide range of nutrients on plant-based foods were important for human health. Among the nutrients, iron is one of the most important micronutrients. Increasing the iron content in plant staple food continues to be a target of many scientists around the world, but the success in effort has sometimes under targets[4]. Meanwhile, *P. putida* IFO 14796 was the soil bacteria that expected to survive in the soil for the long term and continue to increase iron absorption rate into the root of the corn plant. The present research aimed to improve iron contents in maize grain through *P. putida* IFO 14796 intervention. The advantages of this strategy were environmentally friendly, simple in technologically and economically.

MATERIALS AND METHODS

Location and time duration: *Pseudomonas putida* IFO 14796 (Osaka, Japan collection) was accessed from Food and Nutrition Laboratory of Gadjah Mada University, Indonesia. The experiment was under taken for 90 days from September 7th, 2018-December 5th, 2018 in the green house, the center for integrated laboratory of Dayanu Ikhsanuddin University, Baubau, Southeast Sulawesi-Indonesia.

Experimental materials: The proliferation of bacteria was carried out in potato extract- sucrose[16] broth (PESB) at 28°C. Carrier made from combination green compost and rice bran in size particle <0.5 mm was sterilized in an autoclave at 121°C, a pressure of 15 pounds[17] for 1 h. The top soil for the growth media was taken from the agriculture area in particulate size <1.5 mm. *Pseudomonas putida* IFO 14796 (5.1×10^8 CFU g^{-1}) in carrier 3:1 proportion was used for treatment.

Design experiment: The research was undertaken in the randomized group design, where one group for treatment and one group for control. A poly bag (25×25× 40 cm) contained with unsterilized soil media and it is the top mixed with 5% (v/v) of the carrier enriched *P. putida* IFO 14796 (5.1×10^8 CFU g^{-1}) for the treatment and carrier without *P. putida* control. Each group of the experiment was used about 200 of poly bag which was grown one sterilized corn seed (0.36±0.06 g), respectively. Randomized, 12 of the experiment units were chosen to observe the stem size after 55 days of cultivation as well as maize grain samples (g) were taken from all grain in 12 sample unit after was harvested at 90 days in cultivation or 30 days after pollination (DAP).

Data analysis: Stem size data were analyzed by the paired t-test using the software package SPSS 15.0. While the iron concentration in maize grain was analyzed by the atomic absorbance spectrophotometer (Indonesia national standard 19-286-1992 point 3.3.2) method at the Laboratory of center plantation based industry, Makassar, Indonesia. The percentage of improving iron contents in maize grain was analyzed by comparing the difference iron contents in the treatment and control then multiplied by 100 percent.

RESULTS

Corn plant growth: The mean stem size of the plant with the treatment of *P. putida* IFO 14796 was 65.09 cm higher over the control (52.39) after 55 days of cultivation. Based on the t- test, the treatment was significantly different over the control at $p<0.05$ (Table 1).

Iron contents in maize grain: The iron contents in maize grain that inoculated by *P. putida* IFO 14796 was 10.1117 mg kg^{-1} higher than uninoculated or control (8.5130 mg kg^{-1})

Table 1: Average stem size of the corn plant after the intervention of *P. putida* IFO 14796 for 55 days of cultivation

Sample	Stem size of corn plant	
	Control (cm/plant)	Treatment (cm/plant)
1	41.76	54.20
2	54.32	63.43
3	57.34	50.96
4	48.14	53.38
5	57.46	65.94
6	48.04	73.48
7	50.99	61.07
8	54.32	79.44
9	57.37	48.04
10	43.96	68.45
11	51.18	67.51
12	63.74	74.10
Average	52.39	65.09*

*Means difference is significant at $p<0.05$

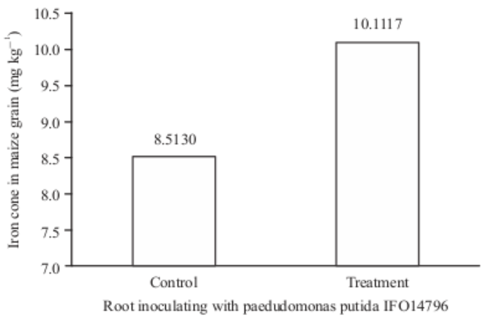

Fig. 1: Iron concentration in maize grain after root inoculating with *P. putida* IFO 14796 for 90 Days

(Fig. 1). Thus, there were improved iron contents in maize grains up to 18.79% after 90 days in cultivated or 30 DAP.

DISCUSSION

Measurement of the corn stem size and mineral content in maize grain intended to provide an overview of the ability of *P. putida* IFO 14796 to enhance the growth and improving the iron ingredient in maize grain product. Average of stem size of corn plant significantly increased after 55 days in cultivated. This phenomenon has increased the expectation that applied *P. putida* IFO 14796 in agriculture able to support corn production. Although the growing corn plant in shoot and root weight, plant height and plant diameter were significantly different from bacterial consortium than over mono-inoculum treatments for blue maize plant[18]. However, the increased stem size of corn plants that were intervened of *P. putida* IFO 14796 was an important outcome to enhance transport minerals from root to leaf and maize grain. Meanwhile, the iron concentration of maize grain with *P. putida* IFO 14796 treatment was 10.1117 mg kg^{-1} higher than control (8.5130 mg kg^{-1}). This illustration revealed that *P. putida* IFO 14796 intervention has caused increasing iron contents in maize grains up to 18.79% after 90 days in cultivated. The previous study also has shown the ability of the *Pseudomonas plecoglossicida* SRI-156 for increasing the iron content in chiaspeak and pigeonpea grains up to 18 and 12%, respectively[19]. However, the transgenic method has increased iron content[20] in rice 3.4-fold and organic and chemical fertilizers intervention has increased iron concentration in wheat grain up to 1.4 fold[21]. The ability of *P. putida* to enhance plant growth and iron content was thought caused by phytohormone and other beneficial compounds production, such as indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins (CTKs), ethylene, extracellular enzymes, antibiotics, cyanide and siderophore which gave a positive effect on plant growth[11, 22].

Biofortification with PGP for increasing the growth and improving iron content in maize grain is very important because iron content in maize grain was 6.2 mg kg^{-1} only[23]. However, the growth and variation of the iron concentration in the grain product was influenced by many factors such as plant genetic, harvest age and mineral content in soil and environment[24], such as iron content in maize grain in sub-saharan Africa-ranged from 15-159 ppm for mid-latitude and from 14 to 134 ppm for lowland maize inbred lines[25]. In the present study, the roots space in the poly bag was thought to be a limiting factor for increasing the growth and iron mineral absorption. Thus, field experiments are needed to maximize the potential of the *P. putida* IFO 14796 to increase the growth and the iron content in the maize grain. The iron content in maize grain resulted from this biofortification process around 10.1117 mg kg^{-1} has a suitable for human consumption as well as previously recommended in rice (6-22 mg kg^{-1}), maize grain (10-160 mg kg^{-1}) and wheat (15-360 mg kg^{-1})[26].

The improved iron concentration in biofortified maize was become fundamental in further research for application the protection motivation theory (PMT) in developing dietary strategies to improve the iron status in iron deficiency anemia (IDA)[27]. As previous study showed intervention of iron biofortified staple food significantly increased serum ferritin concentrations and total body iron[28], iron biofortified pearl millet significantly improved iron status in children[29], iron biofortified bean increased iron status in Rwandan women after 128 days[30] and positively affects cognitive performance in 18-27 year old Rwandan female college students in an 18 weeks[31].

CONCLUSION

From this study it was concluded that *Pseudomonas putida* IFO 14796 with density 5.1 × 10^8 CFU g^{-1} can improve iron content in maize grain up to 18.78% after 90 days in cultivated. Thus, proposed to use iron biofortified maize to develop the research to reduce iron deficiency anemia (IDA) through community trial.

SIGNIFICANCE STATEMENT

This study discovered biofortification through *P. putida* IFO 14796 intervention has increased iron content in maize grain that can be beneficial to overcome iron requirement in human. This study will help the researchers to uncover the critical areas of the role important of *P. putida* IFO 14796 in biofortification for improving iron content in maize grain that many researchers were not able to explore. Thus a new theory on the protection motivation theory (PMT) to reduce the burden of iron deficiency anemia may be achieved.

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