Protein hydrolysates enhance germination and early growth of maize and sugarcane

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Abstract. Protein hydrolysates have been reported as a plant biostimulant due to their activity like phytohormones. This research aimed to study protein hydrolysate's effect on the germination and growth of maize and sugarcane. Chicken feather meal (TB) and trash fish meal (TI) based protein hydrolysates were tested their ability to promote rooting and shooting in the early growth of maize and sugarcane, following an adequately dipping. In the maize bioassay, seeds were soaked for 1 hour in aquadest and 10 ppm of TB and TI hydrolysates in three replicates. Shoots and roots length were measured 5 days after germination. In the sugarcane assay, setts having a single bud on top and bottom stalk of PSJT-941 were grown in a polybag. The experiment was performed in eight treatments and 10 replicates, comprising setts from top and bottom, followed by 5, 10, and 30 minutes dipping in 20 ppm of TB hydrolysate. The setts soaked in aquadest was the negative control. Maize treated with TB hydrolysate had the highest shoots and roots length, while sugarcane setts from the top stalk soaked for 30 minutes in the TB hydrolysates showed germination in all replications besides the best in rooting and number of shoots.

Keywords: phytohormone activity, germination, maize, sugarcane

1. Introduction

Protein hydrolysates are considered a subgroup of plant biostimulants that exhibit phytohormones-like activity. Protein hydrolysates are composed of a mixture of polypeptides, free amino acids and may contain macro and micro-nutrients, polysaccharides, and lipids available in the initial materials [1, 2]. Foliar application or soil-drench was reported to promote secondary metabolites production in response to biotic or abiotic stress and also induce a series of physiological and morphological responses by mimicking growth regulator actions [3]. Auxin-, cytokine-, and gibberellin-like activities were present in the early growth of maize and tomato cutting which promote plant growth, including improvement of root and leaves dry weight, the total dry biomass, leaf nitrogen content, and also enhanced nitrogen uptake by modulating the crop root system architecture and consequently reduce the supply of mineral fertilizers with high crop performances [1, 4-7]. Other research revealed the adventitious rooting enhancement by vegetal-derived protein hydrolysate in several plant cuttings in response to brassinosteroid-mediated processes [1].

Protein hydrolysate applications are capable of improving physiological processes in crops that stimulate growth, such as enhancing yield and product quality [8,3], promoting tolerance to environmental stresses, including drought [9], salinity [10], thermal [11], nutrient stress [12], and adverse soil pH [13]. Protein
hydrolysate has been developed as a plant biostimulant from many sources, such as alfalfa plants, red grape (RG) [3], meat flour [5], soybean [4], tomato plant [14], fish meal, collagen, and keratin [15]. A previous study [16] reported that protein hydrolysates obtained from chicken feather meal (TB) and trash fish meal (TI) have proven to rejuvenate old sugarcane callus, accelerate and improve shoot regeneration, and also reduce browning occurrence in in vitro sugarcane culture.

Protein hydrolysates have been applied as plant biostimulants in various food crops and horticulture, such as winter wheat, corn, soybeans, and cucumbers [17-21]. However, little has known the role of protein hydrolysates derived from chicken feather meal and trash fish meal in plantation crops. This research aimed to study the effect of protein hydrolysates on the germination and vegetative growth of maize and sugarcane.

2. Material and method

2.1. Materials

This research was carried out at Biochemistry and Molecular Biology Laboratory, Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) for five months from August to December 2019. Protein hydrolysates were produced from trash fish meal (TI) and chicken feather meal (TB) under high temperature and pressure using acidic hydrothermal treatment (HTT) as reported by Fitriyah (2019) [6]. The bioassay in corn was performed using commercial corn seed variety of Pertiwi 3 and the seedcanes used in this study was PSJT-941 cultivar sourced from Research & Development unit, PT. PG Rajawali II (PUSLITAGRO) Jatitujuh.

2.2. Bioassay in corn and sugarcane

Two different biostimulants obtained from the hydrolysis of chicken feather meal (TB) and trash fish meal (TI) were tested their ability to promote rooting and shooting in the early growth of maize and sugarcane, following an adequately dipping.

In the first bioassay, maize seeds were germinated in the influence of TB and TI hydrolysates following the experiment design of randomized complete design with 3 treatments, in 9 replicates, including TB hydrolysate 20 ppm, TI hydrolysate 20 ppm, and aquadest as the negative control. Corn seeds were soaked according to the treatment for 1 hour following the germination in petridish covered with wet tissue paper. The germinated seeds were grown further for 5 days to evaluate the number of roots, shoot and root length.

The second bioassay was performed on sugarcane using single bud setts from the top and bottom stalk, which were originally discarded because they often had low germination rates. The treatment includes a variation of dipping time of set in TB hydrolysate at a concentration of 20 ppm. The variation includes 5, 10, and 30 mins dipping of top and bottom setts before planting in polybag. Each sett was germinated and grown in a polybag in 10 replicates according to the treatments. Aquadest was used to soak the setts for 30 mins in the negative control. The observation was performed 1 month after planting by measuring the germination, shoot length, and the number of shoots of all treatments in the top and bottom setts.

2.3. Data analysis

The number of roots, shoot and root length in maize bioassay was statistically evaluated in one-way ANOVA with Duncan's MRT at $\alpha$ 5%. The sugarcane’s germination rate was not sufficient for statistical analysis, while the shoot length and number of shoots were analyzed using one-way ANOVA with Duncan's MRT at $\alpha$ 5%. The top setts and bottom setts were analyzed separately in ANOVA. The sugarcane root was visually observed and presented as a photo of uprooted seedlings in top setts treatment.
2.4. Chemical analysis
Chemical analysis was performed for TB and TI hydrolysate for amino acid content, including L-serine, L-glutamic acid, L-phenylalanine, L-isoleucine, L-valine, L-alanine, L-arginine, glycine, L-lysine, L-aspartic acid, L-leucine, L-tyrosine, L-proline, L-threonine, and L-histidine. The analysis was performed using Ultra Performance Liquid Chromatography (UPLC).

3. Result and discussion
3.1. Early growth of corn seedling
Chicken feather meal (TB) and trash fish meal (TI) hydrolysates were used as treatment during germination of corn seeds at low concentration of 20 ppm. The early growth of corn seedling was evaluated based on the number of roots, shoot and root length as shown in Table 1.

Table 1. Early growth of maize seedling under protein hydrolysates application.

| Treatment       | Number of Root | Shoot length (cm) | Root length (cm) |
|-----------------|----------------|-------------------|-----------------|
| Negative control| 4.56<sup>a</sup>| 7.93<sup>a</sup>  | 4.77<sup>a</sup> |
| TI hydrolysate  | 7.00<sup>b</sup>| 12.00<sup>b</sup>| 8.44<sup>b</sup>|
| TB hydrolysate  | 14.44<sup>c</sup>| 14.20<sup>c</sup>| 12.56<sup>c</sup>|

*Means with different letters in the same column are significantly different at α = 0.05

The result showed significant differences for the number of roots, shoot and root length in both protein hydrolysates compared to the negative control. TB hydrolysates showed the highest number of roots, shoot and root length with 14.2 cm, 14.44, and 12.56 cm in respective order, followed by TI hydrolysate with shoot length of 12 cm, number of roots of 7, and root length of 8.22 cm. On the other hand, negative control showed the slowest growth expressed by the lowest number of roots, shoot and root length at five days after germination. TB hydrolysate, especially, was nearly twice higher in shoot length, three times fold in the number of roots, and 2.5 times in root length compared to the negative control.

Different types of protein hydrolysates induced evident changes in the shoot and root growth in an exponential curve. Visible shoot and root growth among treatments are shown in Figure 1. A previous study [4] found that protein hydrolysate applications in dwarf pea showed improvement of the shoot length by gibberellin mode of action. A similar report also indicates protein hydrolysates produced from alfalfa and meat flour showed a gibberellin-like and auxin-like activity by improving maize's root and leaf growth and inducing different root architecture with a high number of hair roots [5].

The rooting responses were the most dramatically influenced by TB hydrolysate than shoot growth. Rooting is commonly regulated by auxin, including indole-3-butyr acid (IBA), 1-naphthaleneacetic acid (NAA), or a combination between them. Previous research by Fitriyah [6] reported the effect of chicken feather meal and trash fish meal hydrolysates on root and coleoptile growth of mung bean seedlings. The result indicated improvement of root fresh weight in application of 20 ppm TB hydrolysate by 103.4 mg, compared to the negative control with only 70.4 mg. The higher growth improvement in the application of protein hydrolysates were also reported to be more noticeable in rooting than shooting during the early growth of mung bean. It was proposed that root growth improvement promote nutrient intake for shoot growth [6].
Figure 1. Effect of protein hydrolysates on early growth of maize: (A) TB hydrolysates, (B) TI hydrolysates, (C) negative control (aqua dest), and (D) growth comparison of all the treatments.

3.2. Protein hydrolysate promotes shoot and rooting growth of sugarcane

Sugarcane single-bud-setts used in this study were obtained from the top and bottom stalks at the age of 9 months. Farmers usually utilize middle stalk (around 8-10 nodes) as plant materials and remove 3-5 nodes at the bottom and several young nodes at the tops. The top nodes were mostly discarded due to immature bud, which led to a low germination rate or easily dried out. The suppressing sprouts of lower buds is mostly due to the hormonal effect [22].

Application of protein hydrolysates for setts treatment before planting aimed to promote germination and growth of sugarcane setts. The treatments only used TB hydrolysates in different times of dipping application before planting since TB hydrolysate gave higher growth improvement in the corn seedling bioassay. The germination of sugarcane setts was evaluated by calculating germinated setts per total setts used in the treatment. The shoots during early growth were measured and calculated for shoot length and number of shoots 1 month after planting. The results are presented in Table 2.

Table 2. Early growth of sugarcane under protein hydrolysates application*.

| Dipping time | Top stalk | Bottom stalk |
|--------------|-----------|--------------|
|              | Germination (%) | Shoot length (cm) | Number of shoots | Germination (%) | Shoot length (cm) | Number of shoots |
| 0 min        | 70        | 22.13 a       | 1.10 a          | 50             | 12.25 a           | 0.80 a           |
| 5 min        | 90        | 30.14 ab      | 1.90 ab         | 30             | 8.13 a            | 0.70 a           |
| 10 min       | 70        | 25.71 ab      | 1.40 ab         | 10             | 5.55 a            | 0.30 a           |
| 30 min       | 100       | 40.90 b       | 2.50 b          | 30             | 8.19 a            | 0.60 a           |

*Means with different letters in the same column are significantly different at $\alpha = 0.05$

The germination and growth of setts obtained from the top stalk is improved by the application of TB hydrolysates, especially the highest in 30 minutes dipping. Only after dipping in TB hydrolysates for 30%, setts obtained from the top stalk germinated 100%. The germination, shoot length, and the number of shoots in 5 minutes dipping treatment were higher than in 10 minutes dipping, but did not differ significantly. However, TB hydrolysate application have significantly improved the growth of sugarcane compared to the negative control. The growth of sugarcane setts obtained from the top stalk showed in Figure 2.
Setts obtained from the bottom stalk had lower germination and growth compared to setts obtained from the top stalk in this study. There were no significant differences among different times of dipping treatment and negative control, as presented in Figure 3. Seed dormancy is mainly regulated by abscisic acid (ABA) [23]. As previously reported, TB hydrolysate breaks dormancy and promotes germination of mung bean by gibberellin-like activity [6]. Several hormones induce germination and break the dormancy, including Gibberellins, brassinosteroids, ethylene, and cytokinin [24]. It was assumed that the application of TB hydrolysate at 20 ppm was not enough to break the dormancy of bottom sugarcane buds.

The rooting architecture of sugarcane seedlings obtained from the top stalk were visually observed, as presented in Figure 4. There was no calculation for root growth, but it was visible that TB hydrolysate application promotes root growth in all treatments. However, the highest shoot and root growth achieved by dipping sugarcane setts in TB hydrolysate for 30 minutes.

Figure 2. Growth of sugarcane obtained from top stalk: (A) 0 min dipping in TB hydrolysate, (B) 5 min dipping in TB hydrolysate, (C) 10 min dipping in TB hydrolysate, (D) 30 min dipping in TB hydrolysate, and (E) growth comparison of all the treatments (A-D).
Figure 3. Growth of sugarcane obtained from bottom stalk: (A) 0 min dipping in TB hydrolysate, (B) 5 min dipping in TB hydrolysate, (C) 10 min dipping in TB hydrolysate, (D) 30 min dipping in TB hydrolysate, and (E) growth comparison of all the treatments (A-D).

Figure 4. Rooting system of sugarcane seedlings obtained from bottom stalk under different conditions, A0: 0 min dipping in TB hydrolysate, A5: 5 min dipping in TB hydrolysate, A10: 10 min dipping in TB hydrolysate, A30: 30 min dipping in TB hydrolysate.
3.3. Chemical composition of protein hydrolysates

Protein hydrolysates produced from TB and TI were obtained under high temperature and pressure and utilized acid as a catalysis agent. It was reported that chicken feather meal was composed of 74.39% protein, while trash fish meal contained 47.38% protein [6]. A further study reported that the degree of hydrolysis using the same method in the current study was higher for TB hydrolysate compared to TI hydrolysate, 8.89 and 6.33 respectively [6], which mean that TB hydrolysates contain higher amino acid or peptides than in TI hydrolysate. In the current study, amino acid content was analyzed in both hydrolysates. The result is presented in Table 3 below.

| No | Amino acid      | Content (ppm) | TB   | TI   |
|----|-----------------|---------------|------|------|
| 1  | L-serine        | 697.64        | 94.25|
| 2  | L-glutamic acid | 583.29        | 328.75|
| 3  | L-phenilalanine | 327.40        | 68.82|
| 4  | L-isoleucine    | 244.09        | 62.22|
| 5  | L-valine        | 368.34        | 93.34|
| 6  | L-alanine       | 318.28        | 181.15|
| 7  | L-arginine      | 384.85        | 119.38|
| 8  | Glycine         | 477.09        | 288.21|
| 9  | L-lysine        | 177.16        | 165.58|
| 10 | L-aspartic acid | 252.86        | 141.61|
| 11 | L-leusine       | 445.30        | 120.7 |
| 12 | L-tyrosine      | 177.17        | 51.34 |
| 13 | L-proline       | 532.47        | 171.61|
| 14 | L-threonine     | 284.64        | 84.43 |
| 15 | L-histidine     | 91.70         | 40.40 |

Based on the analysis of the amino acid, it was clearly distinguishable that TB hydrolysates showed that higher protein content and a higher degree of hydrolysis produced higher amounts of amino acids. The low concentration of protein hydrolysate application in this study (10 and 20 ppm) was not enough to act as a fertilizer. Therefore, bioactive peptide and free amino acids content in protein hydrolysates prompted the phytohormone-like activity and regulated the growth of maize and sugarcane in this study. Polypeptides and free amino acids in protein hydrolysates are easily absorbed by leaves and roots and may act as signaling compounds in the regulation of plant growth and development, promoting endogenous phytohormonal biosynthesis [25].

Root morphology determines the plant’s ability to penetrate the soil and absorb nutrients and water from the ground. Sugarcane roots performed an important role in the shoot growth and environmental factors [26]. A previous study reported a bioactive peptide from a soybean meal produced by enzymatic hydrolysis contains root hair promoting peptide (RHPP). A dodecapeptide contains the sequence of glycine-glycine-isoleucine-arginine-alanine-alanine-proline-threonine-glycine-asparagine-glutamine-asparagine-glutamic acid-arginine, promote root growth in plants [27], RHPP promotes rooting system by increasing the number of root hair cells in plant thus improving the absorbance area in rooting system. Such rooting system regulation was different compared to rooting regulation by phytohormones ethylene and auxin [28]. A
previous study reported that brassinosteroid-mediated processes are involved in adventive root growth on the application of vegetal-derived biostimulant in cuttings of basil, tomato, and chrysanthemum [1].

4. Conclusion
In conclusion, TB and TI hydrolysates application in this study was able to promote shoot and root growth of corn during the early stage of growth and improve the germination and growth of sugarcane setts obtained from the top stalk, but not in the sugarcane setts obtained from bottom stalk. The chemical composition of both protein hydrolysates, which underwent the same process, produced different amino acids content, with TB hydrolysate containing higher amino acids than TI hydrolysate. The application of protein hydrolysate as a plant biostimulant for seed or setts treatment is a promising option for sustainable agriculture.

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