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Leaf, Anthocyanin, and Protein Production of *Talinum triangulare* Jacq. Willd with Various Rates of P Fertilizer

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ABSTRACT

The effect of P fertilizer rates on growth and production parameters of waterleaf [Talinum triangulare (Jacq.) Willd] were studied over two months (February-April 2010) in Bogor. Treatments of five P-rates (0, 200, 400, 600, and 800 kg SP-18 ha^{-1}) were laid out in a randomized complete block design with three replications. Generally, the effect of P fertilization in waterleaf was mostly prominent at 8th week after planting (WAP). The increase of P rates increased leaf area, leaf fresh weight, edible part (shoot weight), and protein production; but decreased anthocyanin production. These results suggested that to produce high protein and anthocyanin, waterleaf must be fertilized with 400 kg SP-18 ha^{-1}; and P fertilizer must be applied at least two weeks before planting.

Keywords: inorganic fertilizer, phosphorus, portulacaceae, secondary metabolite

ABSTRAK

Percobaan ini dilakukan untuk mengetahui pengaruh dosis pupuk P terhadap peubah pertumbuhan dan produksi kolesom [Talinum triangulare (Jacq.) Willd]. Percobaan dilakukan di Bogor selama dua bulan (Februari-April 2010). Percobaan disusun menggunakan rancangan acak lengkap dengan tiga ulangan. Perlakuan yang diberikan berupa pemupukan P dengan lima dosis (0, 200, 400, 600, dan 800 kg SP-18 ha^{-1}). Secara umum, pengaruh pemupukan P pada kolesom jelas terlihat pada umur 8 minggu setelah tanam (MST). Peningkatan dosis P meningkatkan luas daun, bobot basah daun, bagian dapat dimakan (bobot pucuk), dan produksi protein; namun menurunkan produksi antosianin. Hasil ini menunjukkan bahwa untuk menghasilkan kandungan antosianin dan protein yang tinggi, maka kolesom perlu dipupuk dengan 400 kg SP-18 ha^{-1}; dan pupuk P harus diberikan sekurang-kurangnya dua minggu sebelum tanam.

Kata kunci: fosfor, metabolit sekunder, portulacaceae, pupuk inorganik

INTRODUCTION

Phosphorus (P) is one of the macro nutrients that needed most by plants. The breakdown or conversion of readily available organic P to inorganic solution P, occurs in most soils, but it is usually too slow to provide enough P for crop growth. It happens naturally and sometimes due to agronomic practices (Marschner, 1995). Ukpong and Moses (2001) stated that the most influential variables for waterleaf production is the availability of phosphorus and organic matter content in soil.

Previous studies showed that fertilization is one of the factors that influence bioactive compounds and waterleaf biomass production (Susanti *et al*., 2008; Mualim *et al*., 2009). Application 15 tons chicken manure ha^{-1} in growing media (soil and rice-hull charcoal mix) would produce high biomass; but reduced total bioactive contents, except alkaloids in the leaves and the tuberous roots of waterleaf (Susanti *et al*., 2008). Furthermore, application of 100 kg SP-36 ha^{-1} and 100 kg KCl ha^{-1} in growing media (soil, rice-hull charcoal, and cow manure mix) led to the high anthocyanin production of waterleaf (Mualim *et al*., 2009). However, these studies do not provide information on the single effect of P fertilization to the production, phytochemicals, and phytonutrients content of waterleaf. Therefore, it is necessary to conduct a study that provides information about the effect of P fertilization on waterleaf production and also the anthocyanin and protein contents.

MATERIALS AND METHODS

This study was conducted at IPB Leuwikopo Research Station, Darmaga, Bogor. Location of the research station is at ± 190 m above sea level. The study began in February to April 2010. Anthocyanin and protein content analysis from the waterleaf edible part was conducted in the Laboratory of Chromatography and Plant Analysis, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB.

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The materials used were stem cuttings of waterleaf, growing media (soil), rice-hull charcoal, polybag (size 40 cm x 50 cm) with a capacity of 10 kg of dry soil, urea, KCl, and SP-18. Hitachi U-2001 double beam spectrophotometer (Japan) was used for spectrophotometric analysis.

The experiment was arranged in a randomized block design with single factor. Five rates of P fertilization (0, 200, 400, 600, and 800 kg SP-18 ha^{-1}) were given as treatments and repeated 3 times, so there were 15 experimental units. Each experimental unit consisted of 10 plants so that there were 150 plants. The data that arranged in the randomized block design was subjected to analyze using ANOVA with PROC GLM model at 95% confidence level. Post-hoc analysis was carried out using Duncan multiple range test (DMRT, α = 5%).

Stem cuttings was planted in polybags and the composition of the media used for planting is soil : rice-hull charcoal (3:2, v:v) (Susanti *et al*., 2008; Mualim *et al*., 2009). Basic fertilizer given was urea (N source) and KCl (K source) at 100 kg ha^{-1} (0.5 g polybag^{-1}). SP-18 (P source) was given according to the treatment, i.e., 0, 200, 400, 600, and 800 kg SP-18 ha^{-1} (0, 1, 2, 3, dan 4 g SP-18 polybag^{-1}). According to the rate of each treatment, fertilizer was mixed and diluted with water (2,400 mL). Later, as much as 240 mL fertilizer solution were given to the crop in each polybag at the time of planting. During this research, regular watering was done when necessary and weeding was done manually.

Observation was conducted on production and growth components of waterleaf, i.e., leaf area, leaf fresh weight, edible part (shoot weight), anthocyanin content and production, and protein content and production. The weight of edible part is weight of the waterleaf shoots that were harvested approximately 15 cm from the upheld leaf tip. This part regarded as a marketable part (Susanti *et al*., 2008; Mualim *et al*., 2009). Leaf fresh weight was measured by weighing the fresh weight of whole leaves from the waterleaf canopy. Total anthocyanin assay was estimated according method by Sims and Gamon (2002); and Lowry method (1951) was used for total protein assay. Destructive observations carried out on four plants at 2, 4, 6, and 8 WAP and two plants at the end of the study from each experimental unit.

RESULTS AND DISCUSSION

Results

Leaf Area, Leaf Fresh Weight, and Edible Part (Shoot Weight) of Waterleaf under P Fertilization Treatments

Various P fertilizer rates treatment had no effect on leaf area and leaf fresh weight. P fertilization only significantly affected the edible part (shoot weight) at 2nd WAP. In general, the effect of P fertilizers can be seen by the increasing slope of the regression line. Increased leaf area, leaf fresh weight, and edible part (shoot weight), due to higher rates of P fertilizer occurred at 8th WAP. P fertilization on waterleaf aged less than 8th WAP gave the same effect; and resulting regression line that tended to become horizontal at various rates of fertilizer (Figure 1A, B, and C).

Leaf area, leaf fresh weight, and edible part (shoot weight) of waterleaf at 8th WAP had the same increasing linear regression line, i.e. y = 1.668 x + 19.76 (R^2 = 0.739); y = 0.034 x + 97.85 (R^2 = 0.483) , and y = 0.021 x + 96.67 (R^2 = 0.286), respectively. Thus, P fertilization with 800 kg SP-18 ha^{-1} at 8th WAP produced the highest average leaf area, leaf fresh weight, and edible part (shoot weight).

Total Content and Production of Anthocyanins and Protein of Waterleaf under P Fertilization Treatments

All rates of P fertilizer given has no effect on total content and production of anthocyanin, as well as total content and production of waterleaf protein. P fertilization affects only anthocyanin production at 2nd WAP. In general, the regression line showed a decrease in total anthocyanin content due to higher rates of P fertilizer, but the protein total content increased. The same pattern is also found in anthocyanin and waterleaf protein production (Figure 2A, B, C, and D).

The total anthocyanin content and production increases followed by a decrease in protein total content and

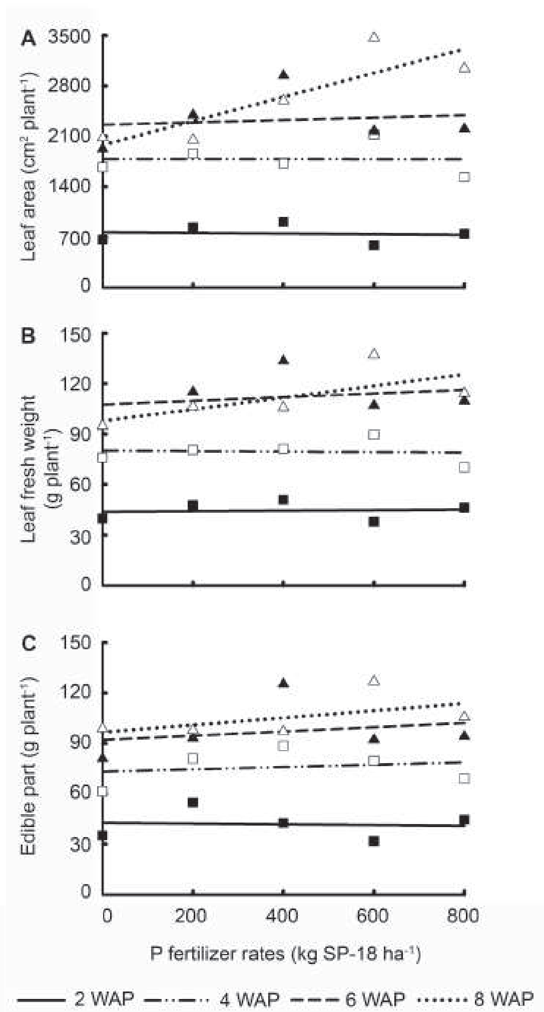

Figure 1. Effect of P fertilization on (A) leaf area, (B) leaf fresh weight, and (C) edible part (shoot weight) of waterleaf

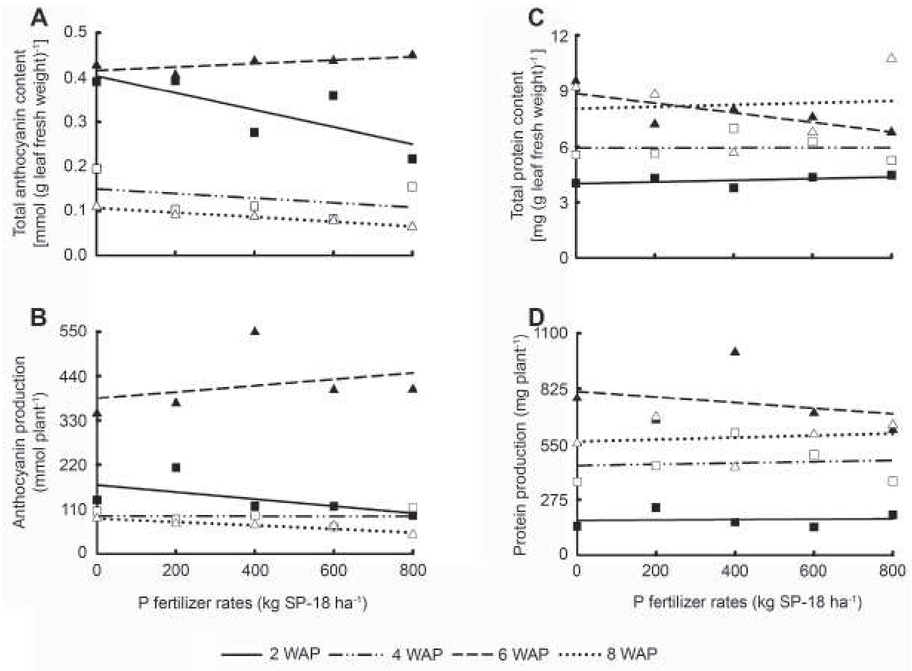

Figure 2. Effect of P fertilization on (A) total anthocyanin content, (B) anthocyanin production, (C) total protein content, and (D) protein production of waterleaf

production occurs at 6th WAP. Increased total anthocyanin content and production each following equation y = 4E-05x + 0.415 (R^2 = 0.548) and y = 0.078x + 384.7 (R^2= 0.098). In contrast, decreased total content and production of protein each following the equation y = -0.002x + 8.850 (R^2 = 0.586) and y = -0.139x + 809.8 (R^2 = 0.086).

Discussion

Effect of P on Leaf Area, Leaf Fresh Weight, and Edible Part (Shoot Weight) of Waterleaf

In general, there was an increase in leaf area, leaf fresh weight, and edible part (shoot weight) with increasing rates of P fertilizer. P fertilization on waterleaf may enhance plant growth, but its effect was slow so that the application must be made several weeks earlier. This could be seen from the regression line that tends to become horizontal before 8th WAP (Figure 1A, B, and C). According to Brown and Courtin (2003) the increasing of plant growth due to the increasing of P uptake so that the leaf area increased. In addition, application of P could increase water use efficiency in crops. Crop responses to P fertilizer that were less visible may also be caused by waterleaf that actually needed little P fertilization. By 2-6 WAP, component of variables did not differ between various rates of fertilization; this could be seen by the regression line that is horizontal. Some studies in other commodities, such as spinach, cabbage, and strawberries are also showing that administration of P fertilizer had no effect on production. This may be due to the varieties used that are not responsive to P fertilization (Wang and Li, 2004; Moor *et al*., 2009). The results of the initial soil analysis showed P available content was very high (61.4 ppm; extract Bray 1) and soil was relatively acidic with pH 5.1 (water extract). This may lead to the inhibited P availability. Furthermore, it was argued that P fertilization did not affect the observed variables when administered to the soil with high P content (Gervais, 2009), and any increase in rate of P fertilizer only linearly increase crop production (Susila *et al*., 2008).

Effect of P on the Total Content and Production of Anthocyanin and Waterleaf Protein

Increasing rates of P fertilizer caused a total anthocyanin content decreased, whereas protein content increased (Figure 2A and C). It can happen due to competition for resources in the form of a precursor which was aromatic amino acids (e.g. tyrosine, tryptophan, and phenylalanine). These precursors were used for proteins or other phenolic compounds synthesis; if waterleaf synthesized more protein then the anthocyanin synthesis (as part of the phenolic group) would decrease.

Anthocyanin content decreased when subjected to the high amount of P fertilization led to the inhibition of anthocyanin synthesis. Research on *Arabidopsis thaliana* showed that inorganic P limitation induced anthocyanin accumulation (Peng *et al*., 2008). Anthocyanin synthesis could be triggered by various biotic and abiotic stress, i.e., pathogen attacks, injuries, UV light, low temperatures, and heavy metal contamination (Steyn *et al*., 2002; Gould, 2004). Thus, waterleaf anthocyanin synthesis often occured on soil with nutrient poor conditions.

Increasing rates of P fertilizer led waterleaf more active in synthesizing protein (Figure 2C). Other studies on different commodities also showed similar result (Polat *et al*., 2007; Dasci *et al*., 2010). Several studies had reported that application of P fertilizer at 38-40 kg P ha^{-1} produced high protein content in legume (Magani and Kuchinda, 2009; Niri *et al*., 2010).

Observations on waterleaf at 6th WAP showed a slightly different pattern, where there was an increase in anthocyanin contents (Figure 2A) but the protein content decreased (Figure 2c). It was probably at the age when crops experienced environment stress thereby affecting the path of metabolism. The average rainfall at the time was 13.89 mm week^{-1} and an average irradiation length was 5.01 hours week^{-1}. Other abiotic factors observation was not performed in this study. The anthocyanin accumulation in the leaves causing the plant to respond quickly towards environmental variations that occured, however this process occured only temporarily (Chalker-Scott, 2002). Thus, waterleaf is responsive towards environmental changes.

Waterleaf anthocyanin and protein production was the result of multiplying the content with the weight of edible part (shoot weight) (Figure 2B and D). However, this study could not found the optimum P fertilizer rate that could produce high portion of edible part, protein, and anthocyanin. This research suggested that to produce high anthocyanin and protein, waterleaf must be subjected to P fertilization at 400 kg SP-18 ha^{-1}. P fertilization that exceeded the rates would decrease the anthocyanin production, but protein production increased.

CONCLUSIONS

Increasing rates of P fertilization, especially at 8th WAP lead to increased leaf area, leaf fresh weight, edible part (shoot weight), and protein production; but decreased anthocyanin production. Furthermore, to obtain high protein and anthocyanin production, waterleaf should be fertilized with 400 kg SP-18 ha^{-1}, and P fertilizer should be given at least two weeks before planting.

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