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Protein and Anthocyanin Production of Waterleaf Shoots (*Talinum triangulare* (Jacq.) Willd) at Different Levels of Nitrogen+Potassium and Harvest Intervals

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ABSTRACT

The experiment was conducted at IPB Experimental Station, Leuwikopo, Dramaga, Bogor, from November 2009 until February 2010 to study the effect of different nitrogen+potassium rates and harvest intervals on protein and anthocyanin production of waterleaf shoot (Talinum triangulare (Jacq.) Willd). A randomized complete block design was used with three replications of two factors, which were four N+K dosages (50 kg urea + 50 kg ha^{-1} KCl, 50 kg urea + 100 kg ha^{-1} KCl, 100 kg urea + 50 kg ha^{-1} KCl, 100 kg urea + 100 kg ha^{-1} KCl) and three harvest intervals (30, 15, and 10 days). The results showed that interaction of 100 kg urea + 100 kg ha^{-1} KCl and 15-day harvest interval produced the highest content (8.29 mg g^{-1} fresh weight) and production (4.72 g plant^{-1}) of protein. The interaction of N+K dosages and harvest intervals were not significant in affecting the anthocyanin content. The highest production of anthocyanin was produced by single treatment of 100 kg urea + 100 kg ha^{-1} KCl (152.23 µmol plant^{-1}) and 10 days harvest interval (165.47 µmol plant^{-1}), respectively. Leaf protein levels negatively correlated with anthocyanin content.

Keywords: anthocyanin, fertilizer, harvest interval, protein, Talinum triangulare

ABSTRAK

Percobaan untuk mempelajari pengaruh berbagai dosis pupuk nitrogen+kalium dan interval panen terhadap produksi protein dan antosianin pucuk kolesom (Talinum triangulare (Jacq.) Willd) telah dilaksanakan di Leuwikopo, Dramaga, Bogor pada bulan November 2009 sampai Februari 2010. Percobaan menggunakan rancangan acak kelompok lengkap dengan 2 faktor dan 3 ulangan. Kedua faktor tersebut adalah dosis pupuk N+K (50 kg urea + 50 kg KCl ha^{-1}, 50 kg urea +100 kg KCl ha^{-1}, 100 kg urea + 50 kg KCl ha^{-1}, 100 kg urea +100 kg KCl ha^{-1}) dan interval panen (30, 15, dan 10 hari). Hasil menunjukkan bahwa interaksi perlakuan antara dosis pupuk 100 kg urea +100 kg KCl ha^{-1} dan interval panen 15 hari menghasilkan kandungan (8.29 mg g^{-1} bobot basah) dan produksi (4.72 g tanaman-1) protein pucuk kolesom tertinggi. Interaksi perlakuan antara dosis pupuk N+K dan interval panen tidak berpengaruh terhadap kandungan antosianin. Produksi antosianin pucuk kolesom tertinggi dihasilkan oleh masing-masing perlakuan 100 kg urea+ 100 kg KCl ha^{-1} (152.23 µmol tanaman^{-1}) dan interval panen 10 hari (165.27 µmol tanaman^{-1}). Kandungan protein pucuk berkorelasi negatif dengan kandungan antosianin.

Kata kunci : antosianin, kolesom, interval panen, protein, pupuk

INTRODUCTION

Waterleaf (*Talinum triangulare*) is a succulent vegetable mostly grown for their edible shoots. Acute toxicity test on waterleaf shoots has demonstrated that the shoots are safe and edible (Nugroho, 2000). Waterleaf shoots have been described to have a medicinal properties and high levels of antioxidants which are important for human health. Waterleaf shoots contain protein (Mensah *et al*., 2008), consisted of 18 amino acids, the highest content being glutamic acid and leusin (Fasuyi, 2006). In addition, waterleaf have been reported to contain flavonoids, steroids and alkaloids (Susanti *et al*., 2008). Mualim *et al*. (2009) further demostrated that one of the detected flavonoids is anthocyanin. Anthocyanin is an important plant pigment that has antioxidant properties (Castañeda-Ovando *et al*., 2009). Antioxidant properties from waterleaf extracts have been demonstrated to enhance the cognitive ability and have benefiting effects on the neurons of the cerebrum (Ofusori *et al*., 2008).

Waterleaf shoot production and their bioactive compounds might be increased by fertilizer application. Potassium is one of the limiting factor to anthocyanin production in waterleaf shoots (Mualim *et al*., 2009). Application of 100 kg of Urea and 100 kg ha^{-1} KCl resulted

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in a highest anthocyanin production compared to application of nitrogen and phosphorus, or potassium and phosphorus. However, the optimum doses of nitrogen and potassium fertilizer application to obtain the highest level of protein and anthocyanin is yet to be determined.

Under proper cultural management waterleaf shoots can be harvested several times (Sugiarto, 2006) at about 2 months after planting (Fontem and Schippers, 2004). Harvest intervals affected biomass production, nutritional value, regrowing potential of the plants after (Man and Wiktorsson, 2003). Li and Strid (2005) reported that harvesting the shoots of *Arabidopsis thaliana* can increase anthocyanin contents of the rosette leaves, whereas extending the harvest interval of *Napier grass* and *Cratylia argentea* decreased protein content (Manyawu *et al*., 2003; Sanchez *et al*., 2007). Very limited studies have been conducted on factors affecting protein and anthocyanin content of waterleaf.

The objective of this study is to determine optimum doses of nitrogen and potassium and harvest intervals to produce high level of protein and anthocyanin in waterleaf shoots.

MATERIALS AND METHODS

Experiments were conducted between November 2009 to February 2010 at IPB's Experimental Station at Leuwikopo, Bogor. Soils in this area are classified as loamy clay, pH 6.90 and had medium CEC of 16.46 me (100 g)$^{-1}$. The average rainfall during the experiment was 344.48 mm month^{-1} with the average temperature of 25.68 ^{o}C and relative humidity of 84.75% .

The experiments used randomized complete block design with two treatments, i.e. doses of nitrogen and potassium of 50 kg urea + 50 kg ha^{-1} KCl, 50 kg urea + 100 kg ha^{-1} KCl; 100 kg urea + 50 kg ha^{-1} KCl, and 100 kg urea + 100 kg ha^{-1} KCl; and harvest intervals of every 30, 15 and 10 day (Table 1). Every treatment is replicated three times so in total there are 36 experimental units of 10 plants for each unit. Data were analysed with ANOVA. Further test used Duncan Multiple Range Test at α = 5%, whereas correlation test analysis used Pearson's method.

Plant materials used were 10 cm cuttings with 2 mature and unfolded leaves, taken from healthy stock plants maintained in a nursery. Cuttings were planted in 10 kg polybags of 40 cm x 50 cm, using mixtures of soil, charcoal of rice hulls (3:2/v:v) and chicken manures of 25 g per *polybag* or equivalent to 5 ton ha^{-1}, mixed two weeks before planting. Nitrogen and potassium were applied by mixing them thoroughly at transplanting date, i.e. when plants are 7 days old. SP-18 fertilizer was applied at 50 kg ha^{-1} for all treatments. Plants were watered daily in the morning, unless there was rain. Ten-cm of shoots, measured on unbended leaves from bases to tips, were harvested from every branch.

Scoring was conducted on fresh weights of marketable shoots, i.e. the accumulated total of shoots fresh weight harvested for the period of 80 days. The protein content was analysed using Lowry's method with Bovin Serum Albumin's standard curve (Waterborg, 2002). The anthocyanin content was analysed using methods developed by Sims and Gamon (2002). Samples were taken from extracts of fresh marketable shoots harvested during 80 days. Protein and anthocyanin production were calculated by multiplying the total fresh weight of marketable waterleaf with their total protein or anthocyanin content.

RESULTS AND DISCUSSION

The greatest fresh weight of waterleaf shoots was obtained from plants fertilized with 100 kg urea + 100 kg ha^{-1} KCl that were harvested every 15 days (Table 2). Plants applied with 100 kg urea + 50 kg ha^{-1} KCl had a similar fresh weight with those applied with 100 kg urea + 100 kg ha^{-1} KCl, which indicated that an increase in nitrogen fertilizer increased vegetative growth of waterleaf regardless of the doses of potassium. Kanzikwera *et al*. (2001) stated that an increase in nitrogen and potassium to certain levels were required to promote vegetative growth and to delay leaf senescence. Nitrogen interacted with potassium in stimulating plant regrowth through increasing the activities of cytokinin as well as other phytohormones that influencing plant growth.

Harvest intervals of 30 days at the same doses of N + K fertilizer resulted in a less weight of harvested shoots compared to those from harvest intervals of 15 and 10 days. It is suspected that the plants harvested at longer intervals reached generative phases and flowered earlier. All plants harvested every 10 days produced harvestable shoots up to 60 days after planting. This treatment might be too intensive and had caused the plants to die earlier, possibly due to their depleted carbohydrates reserves and the decrease in overall plant health. Pruning causes wounds to the plants and the plants will need sufficient period of time to recover and regrow (Kabi and Bareeba, 2008). Similarly, Fontem and

Table 1. Waterleaf harvesting schedule during 80 days harvesting period

Harvest interval (day)	20	30	35	40	50	60	65	70	80
	……DAP (days after planting) ……								
30	√				√				√
15	√		√		√		√		√
10	√	√		√	√	√		√	√

Note : √ = harvest day

Table 2. Total weight of marketable waterleaf shoots at different doses of N+K fertilizer and harvest intervals

Fertilizer doses urea+KCl (kg ha^{-1})	Harvest interval (days)		
	30	15	10 *
	............g $plant^{-1}$............		
50 + 50	46.27h	74.55cd	68.80de
50 + 100	52.75gh	96.27b	84.54bc
100 + 50	55.12fgh	107.93a	86.29bc
100 + 100	66.49def	112.67a	92.97b

Note: Values in the same column and row followed by same letters are not significantly different at 5% DMRT; * = last harvest was on 60 DAP

Schippers (2004) reported that waterleaf shoot production decreased with the increase of harvest intervals. Shortening the harvest intervals resulted in smaller shoots as well as yellowing of old leaves due to nutrient deficiencies.

The highest leaf protein content was obtained from application of 100 kg or urea + 100 kg ha^{-1} KCl harvested every 15 days (Table 3). These results demonstrated that production and protein content increased with the increase of N and K fertilizer at the optimum harvest intervals. It establishes that N and K are the fundamental elements for the protein synthesis. Campbell and Farrel (2006) reported that nitrogen applied to plant roots will be taken up and metabolized into amino acids, which then formed peptide bonds to synthesise proteins, whereas potassium has important roles in enzyme activation and formation of peptide bonds during the process of protein synthesis.

Plants treated with 100 kg urea + 100 kg ha^{-1} KCl had a decreased protein production if harvest intervals were extended, even though this did not happen in plants harvested every 10 days which produced less protein than plants harvested every 15 days. Intensive harvest exceeded the plants capacity to uptake more N from the soil and to regrow. Therefore short harvest interval of 10 days resulted in a shorter harvest duration than longer harvest intervals.

Waterleaf harvested every 30 days produced less protein than those harvested every 15 or 10 days at the same fertilizer N + K doses. Shorter harvest intervals might have caused the waterleaf shoot tips to become major sinks of assimilates. Longer harvest intervals induced earlier flowering and fruit formation, therefore the assimilates will be partitioned to several sinks. This partitioning might have reduced the total N content and the protein synthesis in the leaves (Manyawu *et al*., 2003; Sarwar *et al*., 2006), as longer harvest intervals might induce earlier maturity, reduce biological activities, lowering total N in the leaves, and reduce lignification within the leaves which resulted in a decrease of protein synthesis.

Higher doses of urea and KCl application increased anthocyanin production (Table 4). Treatmenf of 100 kg urea + 100 kg ha^{-1} KCl produced the greatest leaf anthocyain. There results were similar to those reported by Mualim *et al*. (2009) and that 100 kg urea + 100 kg ha^{-1} KCl were an optimum doses for anthocyanin production, and that potassium is the limiting factor in anthocyanin production (Delgado *et al*., 2006). However, overdose application of potassium might decrease anthocyanin production. Therefore, potassium should be applied with optimum doses of nitrogen.

Table 3. Waterleaf shoot protein content and production at different doses of N+K fertilizer and harvest intervals

Fertilizer doses urea+KCl (kg ha^{-1})	Harvest interval (days)		
	30	15	10 *
	.... Protein content (mg g^{-1} FW)		
50 + 50	4.91ef	5.57cde	3.47g
50 + 100	5.29de	6.11cd	4.04ef
100 + 50	5.22de	7.10b	4.03fg
100 + 100	6.26bc	8.29a	4.02fg
	.... Protein production (g $plant^{-1}$)		
50 + 50	0.68g	1.98def	1.68ef
50 + 100	0.83g	3.24bc	2.40cde
100 + 50	0.86g	3.84bc	2.44cde
100 + 100	1.23fg	4.72a	2.61cd

Note: Values in the same column and row followed by same letters are not significantly different at 5% DMRT; * = last harvest was on 60 DAP; FW = fresh weight

Table 4. Waterleaf shoot anthocyanin content and production at different doses of N+K fertilizer and harvest intervals

Treatment	Anthocyanin content (µmol g^{-1} FW)	Anthocyanin production (µmol $plant^{-1}$)
Fertilizer doses urea+KCl (kg ha^{-1})		
50 + 50	0.33	104.97b
50 + 100	0.34	139.64a
100 + 50	0.35	146.85a
100 + 100	0.33	152.23a
Harvest interval (days)		
30	0.30b	84.58b
15	0.31b	157.72a
10 *	0.40a	165.47a

Note: Values followed by same letters within a column and treatment factor are not significantly different at 5% DMRT. * = last harvest was on 60 DAP; FW = fresh weight

Plants harvested every 10 days had the highest shoot anthocyanin, this was not significantly different from plants harvested every 15 days, whereas plants harvested every 30 days had the lowest anthocyanin. The high anthocyanin production in waterleaf shoots harvested every 10 days might have been caused by more pathogen attacks and the earlier senescence compared to plants harvested at longer intervals. Frequent harvest caused woundings which made the plants more prone to pathogenic infection. Plants harvested every 10 days were suffering from stem rot and root rot starting at 50 days after planting. Similar studies were conducted by Karageorgou *et al*. (2008) who reported earlier senescence and the appearance of violetish waterleaf shoots due to anthocyanins production. Disease-infected plants will produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Once consideration only to be deleterious to plant function, ROS are now believed to play crucial roles as the signals for anthocyanin production (Hatier and Gould, 2008). Anthocyanin is a secondary metabolite produced by plants under stress. Anthocyanin acts to absorp light energy by reducing light quantum in the chloroplasts to slow down the production of ROS which accumulated in the chloroplasts. Anthocyanin produced co-pigmentation complex to protect healthy plant tissues (Kangatharalingam *et al*., 2002).

Shoot protein content negatively correlated with anthocyanin content (39%). This indicated that the increase in protein content will be followed by the decrease in anthocyanin, and vice versa. This negative correlation was possibly due to competition of protein formation with anthocyanin formation during the process of phenylalanine biosynthesis, which are the precursors of protein synthesis (Bragazza and Freeman, 2007).

CONCLUSIONS

Plants applied with 100 kg urea + 100 kg ha^{-1} KCl and harvested every 15 days produced the highest weight of marketable shoots with the highest content of protein. Waterleaf anthocyanin content was not affected by the fertilizer doses. Harvest intervals of 10 days and fertilizer doses of 100 kg urea + 100 kg ha^{-1} KCl resulted in the highest shoot anthocyanin content. Protein levels correlated negatively with anthocyanin levels in the waterleaf shoots.

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