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Research Article

Response of Brazilian spinach (*Alternanthera sissoo*) to propagation planting material and NPK fertilizer application[1]

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ABSTRACT

Alternanthera sissoo is currently propagated using stem or terminal cuttings, since it does not produce fertile seeds. This research aimed to identify the most effective propagation planting material among rooted stump, stem cutting and terminal cutting, as well as their response to the application of NPK fertilizer. The results showed a better performance for stem cutting than for rooted stump or apical cutting. The canopy area was positively related to the longest diameter ($R^2 = 0.92$) and the average of two-way cross-sectional diameter ($R^2 = 0.89$). The number of branches, branch fresh weight, leaf fresh and dry weights did not vary among the planting materials; however, the total number of leaves, branch and root dry weights were higher for stem cutting. Stem cutting is also available in a larger quantity than the other two planting materials when collected from each mother plant. Therefore, it is recommended for optimizing leaf yield in *A. sissoo*. The species positively responded to NPK fertilization, as shown by the increase of the SPAD value for 6 to 12 days after the initial application. Therefore, for maximizing yield, it should be regularly fertilized with NPK every 3 weeks, coinciding with the time that the SPAD index falls back to pre-NPK application levels.

KEYWORDS: Infertile seeds, perennial vegetables, stem cutting, vegetative propagation.

RESUMO

Resposta de espinafre-brasileiro (*Alternanthera sissoo*) ao material de propagação e à aplicação de fertilizante NPK

Alternanthera sissoo é atualmente propagado utilizando-se estacas caulinares ou apicais, uma vez que não produz sementes férteis. Objetivou-se identificar o material de plantio mais eficaz para propagação entre estacas enraizadas, caulinares ou apicais, bem como sua resposta à aplicação de fertilizante NPK. Os resultados mostraram melhor desempenho para as estacas caulinares do que para as enraizadas e apicais. A área do dossel foi positivamente relacionada ao maior diâmetro ($R^2 = 0,92$) e ao diâmetro médio da seção transversal de duas vias ($R^2 = 0,89$). O número de ramos, massa fresca de ramos e massa fresca e seca de folhas não diferiram entre os materiais de plantio utilizados; entretanto, o número total de folhas e a massa seca de ramos e raízes foram maiores para as estacas caulinares. Estacas caulinares também estão disponíveis em maior quantidade do que os outros dois materiais de plantio, quando coletadas de cada planta-mãe. Portanto, são recomendadas para otimizar o rendimento da folha em *A. sissoo*. A espécie respondeu positivamente à adubação NPK, conforme indicado pelo aumento no valor SPAD de 6 a 12 dias após a aplicação inicial. Portanto, para maximizar o rendimento, deve ser fertilizada regularmente com NPK a cada 3 semanas, coincidindo com o momento em que o índice SPAD cai para níveis anteriores à aplicação do NPK.

PALAVRAS-CHAVE: Sementes inférteis, hortaliças perenes, estaquia, propagação vegetativa.

INTRODUCTION

Alternanthera sissoo Hort. is popularly known as Brazilian spinach. This leafy vegetable has not been widely cultivated in Indonesia, despite its agronomic and climatic conditions being similar to the Brazilian ones.

Despite systematically belonging to the *Amaranthaceae* family, the morphology of *A. sissoo* is different from other spinaches. It is considered an evergreen perennial plant, but cultivated *A. sissoo* is commonly treated as an annual vegetable crop. Based on our preliminary observation, quality and yield are better if the *A. sissoo* plants are re-planted every 4 months. *A. sissoo* plants could be considered as a fast-growing leafy vegetable, as long as nitrogen availability is continuously maintained (Alam et al. 2022), and prefer moderate shading (50 %), but it can tolerate both heavier shading and full sun exposure (Toensmeier 2007). It prefers well-drained

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but moist soil; however, it can tolerate moderate drought during the dry season (Tiveron et al. 2012). Alam et al. (2022) reported that *A. sissoo* plants vigorously grow using rich organic matter substrate collected from palm oil mill effluent. Sani & Awang (2021) declared that the application of vermicompost produced using red worm (*Eisenia fetida*) positively affects the growth of *A. sissoo* plants.

Most of the spinach family is grown using seeds, but the *A. sissoo* plant does not produce fertile seeds, yet it is easily grown using stem cutting. This spinach rapidly grows in the lowland tropical ecosystem (Yamamoto et al. 2015). Sommai et al. (2021) recommended to harvest it at 15 to 30 days after planting, for attaining the highest total flavonoid content and better leaf digestibility. *A. sissoo* has also been reported as an excellent source of vitamins, minerals, fibers and other healthy natural substances. In addition, it is less preferred by common garden insects (Hussain & Amir 2022).

This leafy vegetable is currently solely propagated using stem or terminal cuttings from reserved mother plants, since its seeds are typically not viable. A mother plant can be incised into rooted stump, stem cutting and terminal cutting. Thus, the present research was designed to evaluate the performance of *A. sissoo* propagated using these three planting materials, as well as their responses to NPK fertilizer application.

MATERIAL AND METHODS

The research was conducted during the rainy season in a humid tropical climate zone in Palembang (104º46’44”E; 3º01’35”S), South Sumatra, Indonesia, from November 2021 to January 2022. Typical agroclimatic conditions at the outdoor research facilities are displayed in Figure 1.

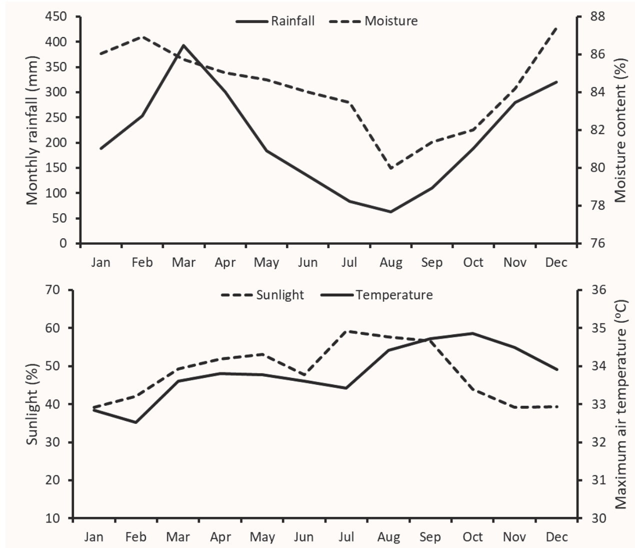

Figure 1. Average typical agroclimatic conditions during 2013-2022 in the research location at the lowland tropics (Palembang, South Sumatra, Indonesia).

Most of the climatic elements do not fluctuate much all year around, except for rainfall distribution. The average monthly rainfall during the last 5 years (2017-2021) reached almost 400 mm during the rainy season and decreased to near 50 mm during the dry season. Despite the fluctuation in the rainfall distribution, the air moisture content rarely falls below 80 %, since two thirds of the Indonesian archipelago are covered by a huge body of water. Sunlight intensity and duration did not change much, and most of the time was partially covered by clouds. The maximum air temperature was considerably high, but for a short period at around midday, it varied from 32 to 35 ºC. The area around the research location is flat, with altitude of less than 10 m above the sea level.

Three different vegetative propagation planting materials from *A. sissoo* plants were used: rooted stump, stem cutting and terminal cutting. Rooted stumps were taken at the base of the stem with roots, including two buds at the leaf axils, but without the leaf blades; stem cuttings at the mid-section of the stem with two leaves included; and terminal cuttings at the tip of the stem including terminal leaf bud.

The planting materials were sown in 30-cm diameter and 30-cm height pots filled with a mixture of soil and chicken manure at a ratio of 3:1 v/v. The soil contained 0.27 % of total N, 720.53 mg 100 g^{-1} of total P_2O_5; 63.94 mg 100 g^{-1} of total K_2O; 123 mg 100 g^{-1} of total Mg; and 1,306.40 mg 100 g^{-1} of available P. The soil pH was 6.63. The commercial chicken manure contained 150 mg 100 g^{-1} of total N; 50 mg 100 g^{-1} of available P; and 2.20 meq 100 g^{-1} of K (Abumere et al. 2019). Based on the low nutrient content in the soil, it was necessary to apply NPK fertilizer: 16 % of total N, 16 % of P_2O_5 and 16 % of K_2O were applied at the dose of 5 g pot^{-1}, at 30 days after planting (DAP).

The pot has four drainage holes at the bottom, and four side holes were made at the position level to the substrate surface for draining the water excess during rainfall, in order to avoid waterlogging.

The collected data were: a) SPAD values, measured starting at one day prior to the NPK application, repeated consecutively every 2 days, and terminated at the 14th day, using a chlorophyll meter (Konica Minolta, SPAD-502 Plus) for monitoring the responses of the *A. sissoo* plant to the NPK application (Lombardo et al. 2020, Lakitan et al. 2021); b) the canopy area was weekly measured using the easy leaf area application for android (Easlon & Bloom 2014), and the canopy index was calculated based on the ratio of the measured canopy area to the projected circular area beneath the canopy, started and ended at 3 and 6 weeks after planting (WAP), respectively. A higher canopy index is associated with dense leaves within the canopy; reversely, a lower canopy index is due to widely scattered leaves within the canopy; c) the maximum and average cross-section of the canopy diameter (CD) were also weekly measured on all plants for studying the correlation between CD and canopy area; d) destructive measurements were carried out at 7 WAP, for collecting data on number of leaves, leaf fresh and dry weight, number of primary and secondary branches, branch fresh and dry weight, shoot and root dry weight, shoot/root ratio and root length. The dry weights of all plant components were measured after being dried in an oven at 100 ºC, for 24 hours. Thicker plant components were thinly sliced (1 mm) prior to the drying process.

The pots were arranged following a randomized blocks design. The treatments were rooted stump, stem cutting and terminal cutting. Each treatment was replicated three times and each replication consisted of five plants. Analysis of variance and significant differences among the treatments were performed using the DSAASTAT for Windows 10 software (Onofri & Pannacci 2014). Furthermore, linear, quadratic, power and exponential regression analysis were carried out to determine the relationship among the selected variables.

RESULTS AND DISCUSSION

The response of the *Alternanthera sissoo* plants to the NPK fertilization rate at 5 g plant^{-1} was exhibited by the increase of the SPAD values after 6 to 12 days of the initial application, depending on the planting materials used (Figure 2). The SPAD value was well established as proxies of leaf chlorophyl and nitrogen contents.

The leaf SPAD values of plants grown using rooted stumps were initially lower than for plants grown using stem and terminal cuttings. However, relatively similar response curves were observed between the rooted stump and stem cutting plants, despite the fact that the rooted stump consistently exhibited a lower SPAD value. Meanwhile, the plants grown using the terminal cutting were only marginally affected by the NPK fertilizer application (Figure 2). The marginal response might be that the additional NPK was over-powered by hormonal activities at the apical meristem.

The leaf SPAD value may be used to determine the response of *A. sissoo* plants to NPK application, since the index is positively and strongly correlated with leaf nitrogen and chlorophyll contents. The strong correlation with leaf nitrogen was proven by Yue et al. (2020) in winter wheat, Hou et al. (2021) in rice and Mendoza-Tafolla et al. (2019) in romaine lettuce, among other similar findings. The nitrogen application resulted in a fast increase in the SPAD value in potato (Lombardo et al. 2020) and soybean (Bobrecka-Jamro et al. 2018). Likewise, a strong correlation with leaf chlorophyl content has been frequently reported, including for wheat (Kumar & Sharma 2019), cassava (Mwamba et al. 2021) and deciduous shrubs (Donnelly et al. 2020). Furthermore, the optimal use of fertilizers increased the leaf chlorophyll content and enhanced the plant photosynthetic yield for Chinese cabbage (Zhang et al. 2022).

In the present study, the SPAD value continuously increased from 6 to 12 days after the

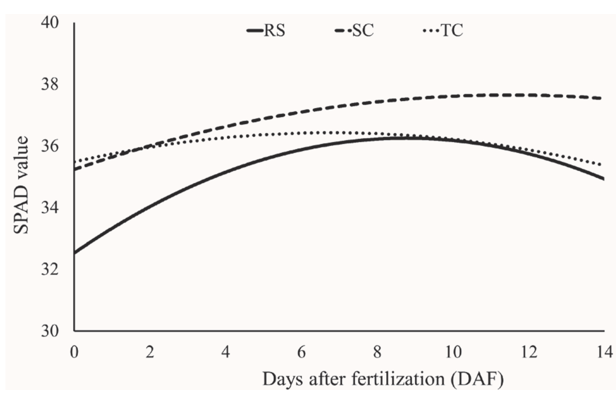

Figure 2. Responses of the *Alternanthera sissoo* plants cultivated using rooted stump (RS), stem cutting (SC) and terminal cutting (TC) to the NPK fertilization, as indicated by the leaf SPAD values.

initial NPK application and dropped back to the pre-application level after 2 to 3 weeks. This information may be used for recommendation on frequency of NPK application in maintaining the growth of *A. sissoo* plants as a fast-growing leafy vegetable. Kartika et al. (2018) reported that the number of tillers in rice is affected by the fertilizer application frequency, and Wu et al. (2019a) found that the split nitrogen application improves the fertilizer and soil nitrogen uptake of pear trees and reduces the nitrogen loss. Meanwhile, Boring et al. (2018) reported that corn grain yields are not responsive to the application frequency, and Aragaw et al. (2020) argued that there is no significant difference for yield between split and regular nitrogen application in sorghum.

The *A. sissoo* grown using rooted stump and stem cutting exhibited similar response curves, despite the fact that the rooted stump plant consistently showed a lower SPAD index. Meanwhile, the plant grown using the terminal cutting was only marginally affected by the NPK fertilizer application. Both the rooted stump and stem cutting have a node with two leaves with opposite positions. However, all leaves were excised in the rooted stump, and a pair of leaves retained in the stem cutting. Each leaf axil has a vegetative bud that potentially grows into a branch. Therefore, during early growth, the rooted stump and stem cutting have benefited from their twin branches, while the terminal cutting grew with a single stem. Lakitan et al. (2021) also reported that a stem cutting with leaves grows more rigorously than a stem cutting without leaves for Javanese ginseng (*Thallium paniculatum*). Similar results were reported for coleus plant. The presence of leaves increases the rooting and sprouting percentage, including number and length of roots (Belniaki et al. 2018).

During the period of 3-6 weeks after planting, an exponential increase of canopy area was observed, regardless of the planting materials used (Table 1); however, the canopy index was consistently higher in plants grown using the terminal cuttings than both the stem cutting and rooted stump plants at early growth (3 WAP), while the differences for canopy index were gradually diminished, and, at the later stage (6 WAP), there were no significant differences among the plants grown using different planting materials (Figure 3).

A higher canopy index was observed in the terminal cutting plant than in the rooted stump and stem cutting plants during the earlier stage (3 WAP), yet the difference was diminished at 6 WAP. The early growth was characterized by the stem elongation of new branches in the rooted stump and stem cutting plants. Meanwhile, the growth in the terminal cutting plant was dominated by the initiation of new leaves that ended up with a denser but smaller canopy size. At 6-7 WAP, the canopy architecture was characterized by short main stem and branches, multiple overlapping leaves and densely populated leaves within the canopy, and culminated in a high canopy index (Figure 4).

The *A. sissoo* canopy was not perfectly round, but densely packed with leaves which contributed to a high canopy index. The wrinkled leaf blade

Table 1. Canopy development pattern during the vegetative phase of the *Alternanthera sissoo* cultivated using different planting materials.

Planting materials	Exponential regression	R^2
Rooted stump	$y = 0.1376e^{0.9957x}$	0.9977
Stem cutting	$y = 0.8168e^{0.7012x}$	0.9850
Terminal cutting	$y = 0.8168e^{0.7012x}$	0.9938

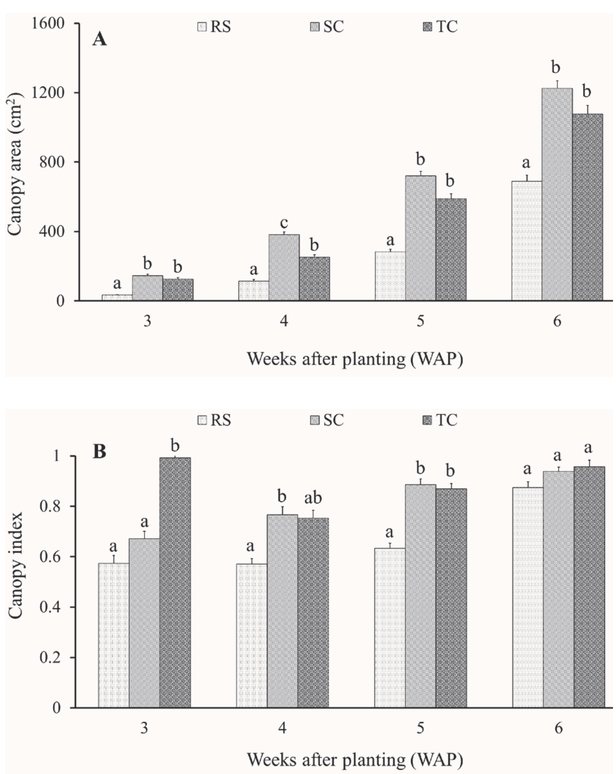

Figure 3. Trends in development of canopy area (A) and canopy index (B) in *Alternanthera sissoo* grown using rooted stump (RS), stem cutting (SC) and terminal cutting (TC) during the early growth stage.

minimized the physical contact among the leaf surfaces, allowing the air circulation and avoiding the complete light blockage within the canopy.

Different approaches have been developed for estimation and direct measurement of canopy density under variable conditions (Dai et al. 2019, Bates et al. 2021). Equal scale visualization for canopy comparison of *A. sissoo* grown using different planting materials at 7 WAP is exhibited in Figure 4, while the correlation between canopy diameter and area is presented in Figure 5.

The *A. sissoo* edible parts are the leaves that can be developed at the main stem, first branch and next-order branches. The optimal harvest was at 7 WAP. The net leaf production was not significantly increased in older plants, since new leaves are compensated with the loss of older leaves. The number of leaves in the first and second branches were comparably similar, but they were significantly different for total fresh and dry weight. The difference in weight was due to heavier and larger individual leaves at the first branch. Despite the higher number and fresh and dry weight of leaves in stem cutting plants, the total leaf fresh weight harvested among the plants grown using different planting materials was not significant (Figure 6). The stem cutting plant also exhibited a higher root dry weight (Table 2) and visually longer roots (Figure 7), when compared to the rooted stump plant.

Photos: Benyamin Lakitan

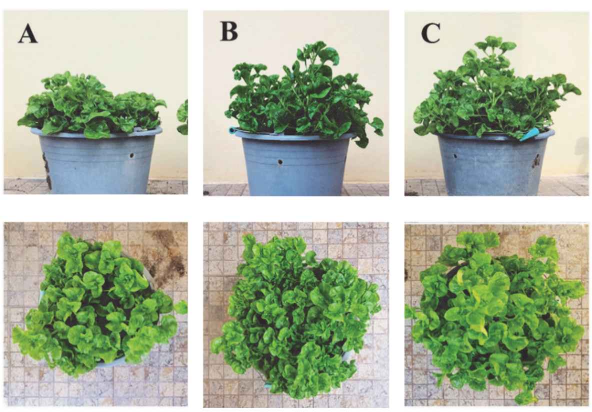

Figure 4. Visualization of the *Alternanthera sissoo* grown using rooted stump (A), stem cutting (B) and terminal cutting (C) at 7 weeks after planting.

The stem cutting performed better than rooted stump or terminal cutting on number of leaves, leaf fresh weight, leaf dry weight, root length and root dry weight, and accumulated more essential nutrients than other planting materials. The accumulated nutrients included nitrogen and carbohydrates required for further growth (Otiende et al. 2017, Cavalcante et al. 2019), respiration process (Collalti et al. 2020) and producing energy for other metabolic processes (O'Leary et al. 2019). Sun et al. (2019) reported that a higher photosynthetic pigment in cucumber leaf was linearly associated with photosynthetic capacity. A similar result was reported for corn by Wu et al. (2019b). The chlorophyll content affects the vegetative plant growth (Wang & Grimm 2021), and the respiration balance in planting material can enhance development and adaptability under stress (Vanlerberghe et al. 2020).

Benbya et al. (2019) concluded that the propagation of *Argania spinosa* using stem cuttings with leaves is not different from stem cuttings without leaves. Meanwhile, *Cannabis sativa* cuttings with

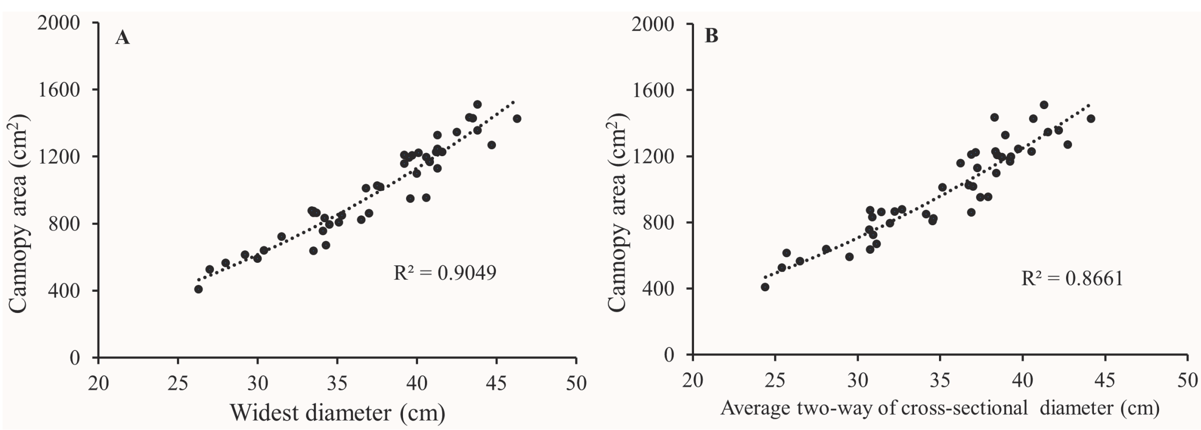

Figure 5. Correlation between canopy diameter and area based on the widest diameter (A) and average two-way cross-sectional diameter (B).

Table 2. Effects of planting materials on shoot and root dry weight of *Alternanthera sissoo*.

Planting material	Shoots dry weight (g)	Root dry weight (g)	Shoot/root ratio
Rooted stump	19.29 ± 0.62 a	1.46 ± 0.07 a	13.48 ± 0.71 a
Stem cutting	31.28 ± 1.75 a	2.22 ± 0.13 b	14.47 ± 1.12 a
Terminal cutting	25.71 ± 1.34 a	1.87 ± 0.15 ab	14.18 ± 0.72 a

Data represent mean and standard error. Values followed by different letters within each column indicate a significant difference at $LSD_{0.05}$.

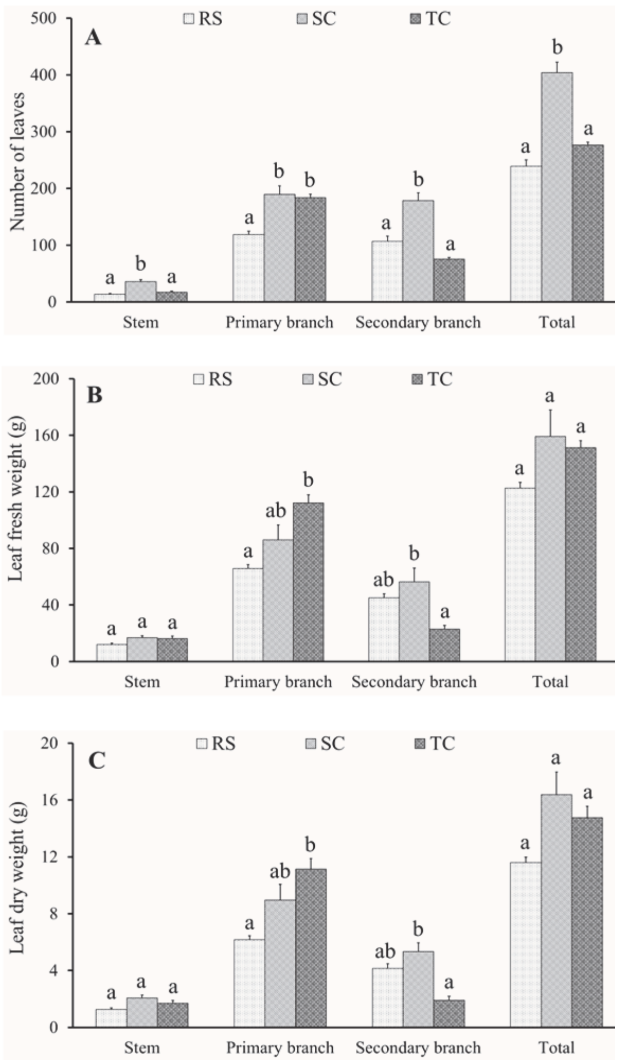

Figure 6. Number of leaves (A), leaf fresh weight (B) and leaf dry weight (C) in *Alternanthera sissoo* cultivated using rooted stump (RS), stem cutting (SC) and terminal cutting (TC) at 7 weeks after planting.

Figure 7. Root length of *Alternanthera sissoo* cultivated using rooted stump (A), stem cutting (B) and terminal cutting (C).

3 leaves showed a better vegetative growth than cuttings with 2 leaves (Caplan et al. 2018). *Santalum austrocaledonicum* with leaves left 100 % had a better root growth than cuttings with leaves at 75, 50, 25 and 0 % (Tate & Page 2018). Meanwhile, the higher photosynthetic pigment increased the antioxidant activity of broccoli (Vicas et al. 2019). An increased photosynthesis leads to an increased biomass accumulation (Qu et al. 2017).

A. sissoo can be propagated using rooted stump, stem cutting or terminal cutting. However, stem cuttings are available in a higher quantity than the other two planting materials when collected from a mother plant. For maximizing harvest, it is recommended to regularly fertilize *A. sissoo* every three weeks. Even though *A. sissoo* is a perennial plant, it is better to do replanting regularly. Further studies should be conducted on searching for optimum time for regular replanting.

CONCLUSIONS

1. Stem cuttings are recommended as planting material in the cultivation of Brazilian spinach (*Alternanthera sissoo*) to obtain a higher leaf yield,

in comparison to the use of rooted stumps or apical cuttings;

2. Brazilian spinach should be regularly fertilized every three weeks with NPK compound fertilizer at a dosage of 5 g plant^{-1} for maximizing its fresh leaf harvest.

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