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Exploring the Growing Environments, Nutritional Properties and Asexual Propagation Method of Ayo/Ariwat (*Tetrastigma harmandii* Planch.)

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Abstract

Tetrastigma harmandii Planch. is utilized for culinary and medicinal purposes in the Philippines. However, its growing conditions, nutritional composition and asexual propagation are less known. Hence, this study characterized the growing environment of T. harmandii in northeastern Cagayan, Philippines and determined its proximate and nutrient compositions through proximate and elemental analyses, respectively. Asexual propagation was also done wherein T. harmandii stem cuttings (one, two and three nodes) with and without auxin (α-NAA) were propagated for 33 days inside the rooting bins. Results revealed that the species grows in various habitats such as hills, coastal areas, residential and agricultural land, grasslands, bodies of water, caves and secondary growth forests in 18-181 masl elevations. It was also found to thrive in moderately acidic soils with medium organic matter and high nutrient contents. The species grows mostly together with Leucaena leucocephala and Gliricidia sepium. Moreover, the leaves of the plant have high crude fiber (5.20%), potassium (3.28%) and iron (207 ppm). The propagation of the plant using three-node stem cuttings without auxin application showed better survival (97.78%) and rooting (86.67%). Compared with one-node stem cutting, improved rooting was observed in the two-node (1.68 more roots and 0.30 denser roots) and three-node (2.92 more roots and 0.14 denser roots) stem cuttings. Hence, T. harmandii is easy to root when propagated asexually; better rooting can be achieved through three-node stem cuttings without auxin application.

Keywords*:* asexual propagation, ayo, habitats, nutritional property, *Tetrastigma harmandii* Planch

1. Introduction

Vitaceae family is well-known for grapes and is ecologically important as major climbers in tropical and temperate forests (Wen *et al.,* 2013). *Tetrastigma* (Miq.) Planch is one of the most species-rich genera of the economically and agronomically important Vitaceae family (Habib *et al*., 2017). *Tetrastigma* is also famous in Southeast Asia for being the host plant of *Rafflesia* (Rafflesiaceae) – a parasitic genus that has the largest flower of all the flowering plants in the world (Wen, 2007; Lianah *et al*., 2015).

Tetrastigma comprised approximately 95 species, which are distributed in Asian tropics and subtropics extending to Australia, from India to China, across Southeast Asia and eastward to Fiji (Wen, 2007; Chen *et al*., 2011; Trias-Blasi *et al*., 2012). It grows suitably in hillsides and valleys of shady and moist primary rainforests (Lianah *et al.*, 2015). *Tetrastigma harmandii* Planch has pedately compound leaves with 3(5)6 leaflets with obscure, flat secondary venation on the adaxial leaf surface, glossy dark green leaflets, simple tendrils, rusty brown globose fruits (Pelser *et al.*, 2016) and ellipsoid seeds (Kochaiphat *et al*., 2016). In the Philippines, Yahya *et al*. (2010) reported *T. harmandii* as the single host plant of *Rafflesia lagascae* (as *R. manillana*) in Mt. Makiling.

T. harmandii, known as 'ariwat' in the Ilocos Region (Vanoverbergh, 1927) and 'ayo' in some parts of the country, is utilized as an indigenous vegetable in the northern part of the Philippines, where it is used in souring Filipino/Ilokano dishes (Maghirang *et al*., 2018). Its sour fruits and leaves are suitable for making preservatives. Although it is utilized as a vegetable, its nutritional content is least reported. As to its medicinal properties, it is known to contain phenols, terpenoids, tannins, saponins (Opeña *et al*., 2021), anti-scabies and diuretic properties (Brown, 1920). It can also treat urinary diseases (Quisumbing, 1951; Carag and Buot, 2017).

Propagation and rooting through nodal stem cuttings have been reported in various plant species such as *Coffea excelsa* (Opeña, 2019), *Arundo donax* (Cavallaro *et al*., 2019), *Dalbergia sissoo* (Husen, 2004), *Pennisetum setaceum* (Cunliffe *et al*., 2001), *Punica granatum* (Naik *et al*., 1999), *Leucaena leucocephala* (Dick *et al.*, 1998), *Acer sp*. (Wilkins *et al.*, 1995), *Rosa sp.* (Stoltz and Anderson, 1987), *Triplochiton scleroxylon* (Leakey and Mohammed, 1985), *Solanum tuberosum* (Goodwin, 1981) and *Dioscorea floribunda* (Chaturvedi, 1975). In *T. harmandii,* propagation has been done through sexual and asexual means by stem cuttings and marcotting (Fern, 2014). However, reports on the propagation and rooting of the *Tetrastigma* species through nodal segments are only limited to *T. hemsleyanum* (Qian, 2008; Du *et al*., 2015), *T. rafflesiae* (Arshad *et al*., 2018), *T. glabratum* (Lianah, 2016) and *T. obtectum* (Yuan *et al*., 2016), while *T. harmandii* stem cuttings were the least reported. Hence, establishing the asexual propagation method of the species will be of great help in the mass propagation and commercialization of *T. harmandii*.

This study aimed to investigate the environmental growing conditions of *T. harmandii* in northeastern Cagayan, Luzon, Philippines and report its proximate and nutritional composition. The study also determined the effects of auxin treatment and varying number of nodal cutting segments on the adventitious rooting of *T. harmandii* stem cuttings.

2. Methodology

2.1 Sampling Site Description and Sampling Method

The presence of *T. harmandii* species in the municipalities of Lal-lo, Camalaniugan, Aparri, Buguey, Santa Teresita, Gonzaga and Santa Ana, Cagayan, Philippines was investigated from June to October 2020. These areas were explored on foot and by riding a vehicle. Photo documentation of the different activities was done. During the conduct of the study, the mentioned municipalities recorded a mean temperature of 25-32 °C, mean rainfall of 224.0-1,014.70 mm and mean relative humidity of 79.0-86.0%.

2.2 Analysis of Environmental Growing Conditions

The habitats where the *T. harmandii* grew and the native and endemic trees/plants that grew within the *Tetrastigma* environment were identified. Soils, where the *T. harmandii* species thrived, were collected from various experimental sites. These were crushed and air-dried at room temperature. The soil chemical properties were analyzed following the procedures described by Motsara and Roy (2008) and the Bureau of Soils and Water Management (2015) for soil pH (potentiometric method), organic carbon/organic matter content (Walkley-Black spectrophotometric method), phosphorus (P) content (Olsen's method), potassium (K) content (cold sulfuric extraction using flame photometer), and copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) contents (diethylenetriaminepentaacetic acid [DTPA] extraction using microwave plasma atomic emission spectroscopy [Agilent MP-AES, Agilent, United States]). Soil test results were interpreted following the rating scales described by Marx *et al.* (1999) and Horneck *et al.* (2011) for total N/organic carbon, total P and total K, and Motsara and Roy (2008) for pH, Cu, Zn, Fe and Mn contents.

2.3 Proximate and Elemental Analyses of T. harmandii

One hundred grams of *T. harmandii* dried leaf tissues from young and mature leaves were collected from Gonzaga, Cagayan and subjected to proximate and mineral analyses. Plant tissue analysis was carried out following the procedures described by Motsara and Roy (2008) and the Bureau of Soils and Water Management (2015) through the Kjeldahl method (crude protein content), filter bag technique (ANKOM, United States) (crude fat and crude fiber contents), titrimetry (calcium [Ca] content), Kjeldahl Jaudber-Gunning method (total nitrogen [N] content), Vanadomolybdate method (total P content), flame atomic emission (total K content) and microwave plasma atomic emission spectroscopy test (Cu, Zn, Fe and Mn contents).

2.4 Asexual Propagation of T. harmandii

Green semi-hardwood *T. harmandii* vines from healthy mother plants were gathered within the vicinity of the Cagayan State University (CSU) – Gonzaga Campus. The rooting experiment was conducted using a completely randomized factorial design (comparing the growth and rooting effects of auxin application and nodal segments in the stem cuttings) in three replications. Each replicate contained 30 *Tetrastigma* stem cuttings, which served as the sample plants. The stem cuttings were allowed to root until 33 days under 'kulob' propagation system or by storing them in an enclosed transparent plastic sheet placed inside the nursery covered with black nets at the College of Agriculture, CSU – Gonzaga Campus (18.24953 °N, 122.00205 °E; elevation: 74.07 masl) from September to October 2020.

Upon collection, the *T. harmandii* plants were propagated through stem cuttings. The green semi-hardwood vines were cut into various nodal segments (one, two and three nodes). The leaves were retained and cut into half of their original length. The basal ends of the stem cutting were also cut and shaped into a bevel. The basal ends of the auxin-treated stem cuttings were submerged in 0.10% α-naphthalene acetic acid solution (0 and 3.70 mll^{-1}) (Philor® α-NAA Plant Growth Promoter, Philor Phil. Orchard Corp., Philippines) for 30 min. After auxin treatment, the whole stem cuttings were quickly submerged for 5 s in fungicide-bactericide solution (2.5 $g\ l^{-1}$)

(Kocide®, DuPont™, Philippines). The stem cuttings were then planted in large-holed seedling trays filled with moist soil media containing three parts of garden soil and one part of vermicompost (pH: 6.25, organic matter: 2.0%, P: 100.10 ppm, K: 413.0 ppm, Zn: 1.06 ppm, Cu: 0.20 ppm, Mn: 3.0 ppm and Fe: 11.20 ppm).

2.5 Rooting Parameters

Rooting success and survival rate were determined using Equations 1 and 2, respectively.

$$\%\ rooting = \frac{Number\ of\ cuttings\ rooted}{Total\ number\ of\ cuttings\ propagated} \times 100 \qquad (1)$$

$$\%\ survival = \frac{Number\ of\ live\ cuttings}{Total\ number\ of\ cuttings\ propagated} \times 100 \qquad (2)$$

Roots per stem cutting were counted when at least 2-mm root protrusion was visible. Root intensity rating was determined using the following scale: 0 = none (Figure 1a); 1 = slight (Figure 1b); 2 = moderate (Figure 1c); 3 = high (Figure 1d) (Opeña, 2019; Opeña *et al*., 2020). Rooting parameters were averaged and the standard error was determined.

Figure 1. Root intensity rating of *T. harmandii* stem cuttings untreated and treated with auxin

2.6 Data Analysis

Data were subjected to two-way analysis of variance (ANOVA) using the Statistical Tool for Agricultural Research version 2.0.1 (International Rice Research Institute). Statistical differences among treatments were determined using the least significant difference (LSD) test at 0.05% level of significance.

3. Results and Discussion

3.1 Growing Environments

T. harmandii was found growing in different habitats of northeastern Cagayan such as lowlands, hills, residential areas, nipa plantations, agricultural lands, grasslands, riverbanks (e.g., Cagayan River), creeks, irrigation dams and canals, swamps and spring, vicinity of caves in Lal-lo and Santa Teresita and secondary growth forests (Table 1).

Table 1. *T. harmandii* growing environments in northeastern Cagayan, Philippines

Growing environments	Description
Habitats	Lowland, hills, residential areas, near coastal areas (approx. 5-200 m away from the sea) and Palaui Island shorelines, nipa plantations, agricultural lands, grasslands; near to rivers, creeks, dams, swamps, springs, and irrigation canals; outside the caves, secondary growth forest/open forests
Elevations	18.90-181.97 masl
Soil chemical properties	pH: 6.05±0.43 Organic matter/organic N: 1.97±1.06% P: 48.73±25.67 ppm K: 451.67±128.69 ppm Zn: 0.82±0.62 ppm Cu: 1.49±0.28 ppm Mn: 23.40±5.37 ppm Fe: 16.80±9.57 ppm
Timber and non-timber species within the growing environment	*Antidesma bunius* (L.) Spreng (Currant tree/‘bignay’) *Antidesma ghaesembilla* Gaertn (Black currant tree/‘arosep,’ ‘binayuyo’) *Areca catechu* L. (Areca nut palm/‘buñga,’ ‘boa’) *Ceiba pentandra* (L.) Gaertn (White silk cotton tree/‘kapok’) *Donax canniformis* (G. Forst.) K. Schum. (Common donax/‘darumaka’) *Eucalyptus globulus* Labill (Blue gum tree/eucalyptus) *Ficus benjamina* Linn. (Weeping fig/‘balete’) *Ficus nota* (Blanco) Merr. (Sacking tree/‘tibig’) *Ficus septica* Blanco (Hauili fig tree/‘hauili,’ ‘ria-ria’) *Ficus ulmifolia* Lam. (‘Isis,’ ‘oplas’) *Gliricidia sepium* (Jacq.) Kunth ex Walp. (St. Vincent plum/‘kakawate’) *Gmelina arborea* Robx. (White teak/gmelina) *Gnetum gnemon* Linn. (‘Bago’) *Harpullia arborea* (Blanco) Radik. (Tulip wood tree/‘uas’) *Hibiscus tiliaceus* Linn. (Sea rosemallow/‘malabago’) *Kleinhovia hospita* Linn. (Timanga tree/‘biknong’) *Leucaena leucocephala* (Lam.) de Wit (Lead tree/ipil-ipil) *Macaranga tanarius* (Linn.) Muell.-Arg (Elephant’s ear/‘binunga,’ ‘samak’) *Melanolepis multiglandulosa* (Reinw. Ex Blume) Reichb. & Zoll. (‘Alim’/‘alem’) *Morus alba* Linn. (Mulberry/‘morera’) *Nypa fruticans* Wurmb. (‘Sasa’/‘nipa’) *Phragmites vulgaris* (Lam.) Trin. (Common reed/‘tambo,’ ‘tanobong’) *Pterocarpus indicus* Willd. (Rosewood/narra) *Swietenia mahogani* (L.) Jacq. (Mahogany) *Syzigium cumini* (L.) Skeels (Java plum/‘duhat’) (‘Dirig’)

n = 3; mean ± std. error

The species was also observed to thrive in low to mid-elevations ranging from 18 to 187 masl and in the coastal areas of Aparri, Buguey, Gonzaga, Santa Ana and in the shorelines of Palaui Island within approximately less than 5 to more than 100-m away from the sea.

Timber and non-timber plant species such as ‘dirig’ or ‘alem’ (*Melanolepis multiglandulosa*), ‘bago’ (*Gnetum gnemon*), ‘samak’ (*Macaranga tanarius*), ‘bignay’ (*Antidesma bunius*), ‘binayuy’ (*Antidesma ghaesembilla*), ‘tanobong’ (*Phragmites vulgaris*), narra (*Pterocarpus indicus*), ‘biknong’ (*Kleinhovia hospita* L.), ‘malabago’ (*Hibiscus tiliaceus*), ‘darumaka’ (*Donax canniformis*), ‘uas’ (*Harpullia arborea*), *Ficus* species and other identified trees flourish within the *T. harmandii* environment. *T. harmandii*’s growth habit is climbing (Mokhtar, 2016), where most of its stems were seen trailing over ‘kakawate’ (*Gliricidia sepium*) and ipil-ipil *(Leucaena leucocephala*).

Moreover, based on the soil chemical property rating scales (Marx *et al.*, 1999; Motsara and Roy, 2008; Horneck *et al.*, 2011), it was shown that *T. harmandii* grows in various soils in northeastern Cagayan with moderate acidity (pH: 6.05), medium organic matter (1.97%), excessive P (48.73 ppm), high K (451.67 ppm), low Zn (0.82 ppm), high Cu (1.49 ppm) and very high Mn (23.40 ppm) and Fe (16.80 ppm) contents (Table 1). This implies that *T. harmandii* burgeons in soils with high nutrients and less acidity.

3.2 Nutritional Content

Proximate and elemental analyses of young and mature leaves showed that the leaves were packed with macronutrients and micronutrients. They contained 3.80% crude protein, 1.37% crude fat, 5.20% crude fiber, 1.99% N, 0.18% P, 3.28% K, 0.30% Ca, 28-ppm Mn, 207-ppm Fe, 41-ppm Zn and 9-ppm Cu (Table 2).

Comparing the nutrient content of *T. harmandii* leaves with that of *Vitis vinifera*’s as reported by Rebolé and Alvira (1986), *T. harmandii* has higher micronutrient contents (59.30-ppm more Fe; 14.81-ppm more Zn; and 4.15-ppm more Cu). However, proximate composition and macronutrient contents are higher in *V. vinifera* than in *T. harmandii*. With its nutrient contents, *T. harmandii* can be a food and feed supplement, as well as plant fertilizer.

Table 2. Nutritional content of leaves of *T. harmandii* Planch. growing in Gonzaga, Cagayan, Philippines

Nutrients and minerals	Test method	Amount (per 100 g sample)	CV (%)
Crude fat	Filter bag technique (ANKOM)	1.37±0.02%	1.93
Crude fiber	Filter bag technique (ANKOM)	5.20±0.12%	3.85
Crude protein	Kjeldahl method	3.80±0.01%	0.66
Total N	Kjeldahl Jaudber-Gunning method	1.99±0.08%	6.57
Total P	Vanadomolybdate method	0.18±0.01%	5.56
Total K	Flame atomic emission	3.28±0.08%	4.40
Total Ca	Titrimetry	0.30±0.01%	3.33
Mn	Microwave plasma atomic emission spectroscopy	28.0±0.58 ppm	3.57
Fe	Microwave plasma atomic emission spectroscopy	207.0±4.33 ppm	3.62
Zn	Microwave plasma atomic emission spectroscopy	41.0±1.15 ppm	4.88
Cu	Microwave plasma atomic emission spectroscopy	9.0±0.06 ppm	1.11

n = 3; mean ± std. error

3.3 Asexual Propagation of T. harmandii Planch. by Auxin Application and Varying Nodal Segments

With the above-mentioned findings on the *T. harmandii*'s nutrient contents, an efficient propagation strategy is needed to reproduce the species for future use. Hence, as stated in the preceding section, a propagation attempt was done, wherein the rooting effects of auxin application and varying nodal segments were investigated to establish a propagation technology for the species. Results demonstrated that the survival rate was improved by propagating the species with stem cuttings containing three nodes without auxin application (97.78%) and two-node stem cuttings with auxin treatment (92.22%). Rooting in the *T. harmandii* stem cuttings can occur even without auxin treatment but was found to improve by increasing to three nodal segments. With the application of auxin, the rooting success in two-node and three-node stem cuttings was reduced by 6.67 and 4.45%, respectively (Table 3).

Table 3. Rooting in *T. harmandii* node stem cuttings with and without auxin (*α*-naphthalene acetic acid) after 33 days in the rooting bins

Rooting treatments	Survival (%)	Shoot growth (%)	Rooting success (%)	Rooting parameters: Number of roots	Rooting parameters: Root length (mm)	Root intensity rating*
Without auxin application						
One node-stem cuttings	87.78^{b}	48.89^{b}	60.0	4.42±0.475	44.31±5.479	1.41±0.128
Two node-stem cuttings	86.67^{b}	80.0^{a}	74.44	7.14±0.690	58.51±5.313	1.74±0.124
Three node-stem cuttings	97.78^{a}	86.67^{a}	86.67	7.63±0.531	52.99±4.234	1.50±0.114
With auxin application						
One node-stem cuttings	85.56^{b}	80.0^{a}	77.78	4.87±0.532	52.52±5.098	1.38±0.116
Two node-stem cuttings	92.22^{a}	63.33^{a}	71.11	5.49±0.540	58.44±6.217	1.66±0.129
Three node-stem cuttings	86.67^{b}	74.45^{a}	73.33	7.49±0.609	65.22±6.222	1.68±0.132

Means with the same letter are not significantly different in LSD test at 0.05 level; *n* = 90; mean ± std. error;
*0 = none, 1= slight, 2 = moderate, 3 = high

Rooting was higher in three-node stem cuttings without auxin application. It was observed that rooting of auxin-treated multiple-node stem cuttings was lower compared with untreated stem cuttings. This may be due to the inhibitory effect of high concentrations of auxin to rooting in the stem cuttings. It is held that the level of endogenous auxin in the stem cuttings was enough to promote rooting. However, with the addition of exogenous auxin to the stem cuttings, the amount of auxin in the stem cuttings became inhibitory for rooting. This observation is consistent with that of De Klerk *et al*. (1997), who reported the inhibition of root growth in vitro apple shoots with the application of supraoptimal auxin (NAA) concentrations. Another possible root inhibitory effect of exogenous auxin application was the ethylene production during adventitious root formation. Bai *et al*. (2020) stated that indole-3-butyric acid (IBA) stimulated ethylene production and suggested that the inhibitory role of auxin on adventitious root elongation in apples was partially mediated by ethylene production. In another study, Alarcon *et al*. (2014) demonstrated the synergistic action of auxin and ethylene on inhibition of root elongation.

The high survival and rooting percentage in three-node stem cuttings without auxin application can be ascribed to more food reserves or carbohydrates contained in the tissues and an adequate amount of endogenous auxin in the plant. Root production of the stem cuttings was reduced with auxin application but was not significantly different from those without auxin application. Stem cuttings with and without auxin were at par in terms of root length (Table 3).

During the rooting observation, the stem cuttings produced shoots (Figure 2). Shoot production was high in stem cuttings with auxin application regardless of nodal segments and in multiple nodal stem cuttings without auxin application. It is perceived that the newly emerged shoots in the stem cuttings have a crucial role in the prolonged survival of the stem cuttings.

Figure 2. Rooted *T. harmandii* one-node (a), two-node (b) and three-node stem cuttings (c) after 33 days in the rooting bins

Leaves from the newly emerged shoot and the ones that were retained from the stem cuttings were assumed to be functioning in photosynthesis even if the stem was already detached from the mother plants. Davis and Potter (1981) reported that the accumulated amount of carbohydrates in the base of the cuttings was correlated with photosynthetic activity. Carbohydrates can also accumulate in the upper portion of leafy cuttings until rooting. In easy-to-root avocado cuttings, the presence of leaves on the cuttings exerts a strong, stimulating influence on rooting whereby after five weeks of propagation, five times more starch in the base of the cuttings was observed (Hartmann *et al*., 2002).

In terms of the number of roots, two-node and three-node stem cuttings significantly produced 1.68 and 2.92 more roots, respectively, than the one-node stem cuttings (Table 4). The adventitious roots of the two-node stem cuttings were also denser and more fibrous than the one-node stem cuttings (Figure 2). Better rooting, observed in the stem cuttings with more nodes, was possibly due to more food reserves in the said stem cuttings. This result was parallel to the findings of Hailemichael *et al.* (2012) and Adugna *et al.* (2015), wherein improved rooting was obtained in multiple nodal cuttings of *Vanilla planifolia*.

Table 4. Rooting response of *T. harmandii* node stem cuttings after 33 days in the rooting bins

Rooting treatments	Rooting parameters	
	Number of roots	Root intensity rating*
One node-stem cuttings	4.64^{b}	1.40^{b}
Two node-stem cuttings	6.32^{a}	1.70^{a}
Three node-stem cuttings	7.56^{a}	1.59^{ab}

Means with the same letter are not significantly different in LSD test at 0.05 level; *n* = 90; *0 = none, 1 = slight, 2 = moderate, 3 = high

4. Conclusion and Recommendation

It was observed that *T. harmandii* thrives in various habitats of northeastern Cagayan such as near coastal areas, bodies of water, caves, forests, residential areas, grassland and agricultural lands, plantations and shorelines of Palaui Island with elevations ranging from 18 to 181 masl. The species can grow in slightly to strongly acidic soils with varying soil nutrients. Out of several plant species, ipil-ipil and 'kakawate' (*G. sepium*) were mostly found growing within the *T. harmandii* environment. The amount of crude fiber, K and Fe was high compared with other nutrients. Hence, *T. harmandii* leaves have the potential use for developing various products such as food supplements, fertilizers and animal feeds. As for the asexual propagation using stem cuttings, survival and rooting were better in three-node stem cuttings without auxin application. Two-node and three-node stem cuttings produced more roots than the one-node. *T. harmandii* stem cuttings were easy to root when propagated asexually. This means that the species can be multiplied without auxin application, and rooting is best with several nodal cuttings.

For future works, it is suggested to evaluate the endogenous auxin and photosynthate or carbohydrate levels of the stem cuttings with increasing nodal segments. This will give insights into the rooting of the species.

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