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Planting Materials, Shading Effects, and Non-Destructive Estimation of Compound Leaf Area in Konjac (*Amorphophallus Muelleri*)

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Abstract

Konjac glucomannan has been commercially produced and used as functional food, food additives, food supplements, pharmaceutical and cosmetic, and biomaterials. Despite intensive and advance research at postharvest stage, knowledge on cultivation of konjac plants has been limited. This research covered current issues associated with selecting agronomically the most suitable planting material, shading effects on shoot emergence and growth characteristics, and non-destructive area estimation of the compound leaf in the konjac plants. Planting materials used were 81 true seeds, 81 bulbils and 81 cormels. Results of this study indicated that bulbil was a suitable planting material based on its early shoot emergence and size of above ground organs. Shading at 50 and 70 % exhibited a better performance in time of emergence and growth characteristics than konjac plant fully exposed to sunlight, even though the differences were not statistically significant. Total leaf blade area (LA) of the irregular konjac compound leaf can be accurately ($R^2 = 0.9932$) and consistently estimated using the 0-intercept linear model and the multiplication product of total midrib length and average width of all leaflets ($TL_M \times AW_L$) is used as predictor. The recommended formula is $LA = 0.6761(TL_M \times AW_L)$.

Keywords: Compound leaf, Light availability, Pseudo-stem, Shoot emergence, Vegetative growth

Introduction

The genus of *Amorphophallus* has about 200 species in the Araceae family, primarily originate from southern, south-eastern, and eastern Asia with few species originate from Africa [1]. The most popular konjac is *Amorphophallus muelleri* Blume. The other edible species are *A. paeoniifolius*, *A. Konjac* K. Koch, and *A. variabilis* [2]. The cultivation of konjac (*A. muelleri*) is not easy but it offers an irresistible challenge for any keen agricultural researcher. It was found that konjac showed positive response to suitable shading, good drainage, and application of manure as an organic fertilizer [3]. Good planting efficiency increased the farmer economic income and their enthusiasm for planting. Area of konjac cultivation has been expanding [4].

Konjac corm is used as food or food additives (e.g., thickener or emulsifier), food supplements for weight management, and in pharmaceutical and cosmetic industries [1]. It is used widely in China and Japan for commercial konjac glucomannan production [5]. Konjac glucomannan is a non-ionic hydrocolloid which has been widely used as food additive or dietary supplement due to its water hold, thickening and gelling capacity [6]. A diet containing konjac flour is considered as healthier since it can reduce the levels of glucose, cholesterol, triglycerides, and blood pressure and weight loss. The health benefits include anti-diabetic, anti-obesity, laxative, prebiotic, and anti-inflammatory. The konjac glucomannan and its derivatives have been applied in bio-technical, pharmaceutical, tissue engineering, and fine chemical fields [7]. Konjac glucomannan incorporated with bionanoparticles has been developed

for active food packaging film [8]. Konjac-based products tend to be biodegradable, biocompatible and non-toxic [9].

Despite multiple uses and high demand of konjac glucomannan and has been cultivated and consumed for a very long time in many regions, cultivation practices of konjac plants have not been well established, especially in the tropical climates. Shenglin *et al.* [10] reported that konjac plant is still grown under semi-wild conditions in many producing areas using unimproved original genetic material in most regions and cannot meet the demand for industrial-scale production. It requires 2 - 3 alternate wet-dry annual cycles of cultivation before konjac cormel reaches its marketable size. Konjac plant is cultivated using either one of 3 types of planting material, i.e., true seed, bulbil, and cormel. Each has different morphological and growth characteristics. However, it is unclear on which planting material is more agronomically beneficial and responses of konjac plant to low light intensity.

Objectives of this research were to compare amongst planting materials and effects of shading on time required to emerge after sowing, shoot growth characteristics, and non-destructive area estimation of the compound leaf of the konjac plants.

Materials and methods

Location and planting materials

The research was carried out from November 2020 to February 2021 in Palembang (104°46'44" E; 3°01'35" S), South Sumatra, Indonesia. The planting materials used consisted of seed, bulbil, and cormel. Average weights of seed, bulbil, and cormel were 0.17, 1.83 and 16.35 g; average of diameters were 4.94, 14.18 and 32.84 mm; and average thicknesses were 4.0, 11.4 and 22.6 mm, respectively.

Research protocol

They were soaked in water for 1 h prior to planting. The pot size used was 30 cm in diameter×30 cm in height. The pots were filled with mixed soil-manure substrate of 5:1 v/v. Fungicide was applied for preventing infestation of soil-borne fungi. Planting materials were placed at depth of 1 cm below substrate surface. Daily watering was applied for maintaining substrate moisture after the planting materials were sown. The konjac plants were fertilized with 25 g Urea, 12.5 g SP36 and 12.5 g KCl after the leaves were fully open, i.e., at 5 weeks after sowing (WAS) for plants grown using true seeds and bulbils and at 7 WAS for those grown using cormel. Fertilizer applications were repeated 4 times at 2 weeks interval.

Experimental design

The experimental design adopted in this research was the split plot design. Shading treatments were assigned as the main plot, consisted of 0, 50 and 70 %. The subplot consisted of the 3 kinds of planting material, i.e., true seed, bulbil, and cormel. The treatment combinations were replicated 3 times and each replication consisted of 3 plants.

Data collection

Data collected include days to visible shoot emergence; length and diameter of the pseudo-stem, midrib length and width of leaflets; number, total blade area, SPAD value, and moisture content of leaflets; fresh and dry weight of pseudo-stem, cormel yield, and roots. Total blade area of each leaf was measured using the LIA13 digital analyzer application. SPAD value was measured using Chlorophyll Meter (Konica Minolta SPAD-502 Plus). Dry weight was attained by setting the oven at 80 °C for 48 h.

Statistical analysis

Analysis of variance and least significant difference for testing significant differences were carried out using statistical analysis software (SAS 9.0 for Windows, SAS Institute Inc., Cary, North Carolina, US). Trend analysis between predictor and estimated leaf area was using linear, quadratic, power, and exponential regressions and predictors used were average of all leaflet length (L_L), average of all leaflet width (W_L), total midrib length of all leaflets (TL_M), and the multiplication product of total midrib length of all leaflets and average of all leaflet width ($TL_M \times AW_L$).

Results and discussion

Effects of shading on shoot emergence of different planting materials

Timing of shoot emergences was significantly different amongst konjac plants grown using true seeds, bulbils, and cormel regardless of shading treatments. Shoot emergence in konjac was significantly earlier if the true seeds were used as planting material, compared to bulbils and cormels. Average time to emergence was 6.1 days for seeds and 8.6 days for bulbils. Moreover, use of cormel in konjac cultivation required extremely long time (38.2 days) to shoot emergence (**Table 1**). It is possible that the planted cormel was at dormant condition since time to shoot emergence was shorten if the cultivation was conducted under 70 % shading. Indriyani and Widoretno [11] reported that lengthening photoperiod to 16 h/day or longer, with light intensity fixed at 400 lux, stimulated dormancy breaking in konjac cormel.

Effect of shading on breaking dormancy may not be direct. Shading reduces light energy receive at soil surface; therefore, soil temperature declines and evaporation rate decreases; therefore, preserved water in soil was higher [12,13]. Dormancy might be broken due to higher soil moisture and/or due to lower soil temperature.

Breaking dormancy by soaking the planting materials in water or chemical solutions had been carried out in many studies, i.e., soaking seeds of konjac for 3 h in KNO_3 solution at rate of 3,000 ppm shortened time to germinate from 3 - 6 months to around 14 days [14]. Other chemicals had been used for breaking dormancy were 6-benzyl amino purine (BAP) at 100 mg/L and ethephon at 300 mg/L [15]. However, some of the efforts had not been effective, for instance, soaking the seeds up to 18 h in gibberellic acid (GA) solution at rates up to 500 ppm did not significantly fasten shoot emergence in konjac plant [16].

Table 1 Emergence of the 1st leaf in true seed, bulbil, and cormel of konjac (*Amorphophallus muelleri*).

Planting material	Shading (%)			Mean \pm SE
	N0	N50	N70	
True seed	6.11 \pm 0.40 a	5.44 \pm 0.11 a	7.00 \pm 0.51 a	6.18 \pm 0.45 a
Bulbil	8.55 \pm 0.80 b	8.00 \pm 0.38 b	8.22 \pm 0.22 b	8.26 \pm 0.16 b
Cormel	38.22 \pm 2.89 c	37.44 \pm 2.99 c	24.55 \pm 4.55 c	33.40 \pm 4.43 c
Mean \pm SE	17.63 \pm 10.32 A	16.96 \pm 10.27 A	13.26 \pm 5.66 A	

Data was presented as Mean \pm Standard error. The means followed by different small letters within each column indicates significantly different among planting materials and the means followed by similar capital letter indicate insignificant different among shading treatments at LSD.05.

Shoot emergence of *A. paeoniifolius* was earlier in whole corms than in the sectioned corms, irrespective of weight. Moreover, smaller cormels emerged earlier than the larger ones [17]. In this study, smaller planting materials (seeds and bulbils) also emerged faster than larger planting materials (cormel). Differences in time of emergence amongst seed, bulbil, and cormel in konjac may or may not be associated with the size differences; rather, it most likely due to different physical and/or physiological characteristics of the planting materials.

Planting materials used significantly affects the morphological characters in konjac plants. Plant grown using cormel and bulbil exhibited much larger size than those grown using true seed. Size differences amongst konjac plants grown using different planting materials were visually distinctive and easily classifiable. Despite its moderately late emergence, bulbil seedlings immediately grew soon after emergence. Height of bulbil-originated konjac plants (BOK) exceeded seed-originated plants (SOK) during the 3rd week after sowing period. Cormel-originated plants (COK) exhibited faster growth than BOK plants. The COK grew faster than the SOK and BOK did. The COK plants attained taller pseudo-stem during the fifth WAS (**Figure 1**).

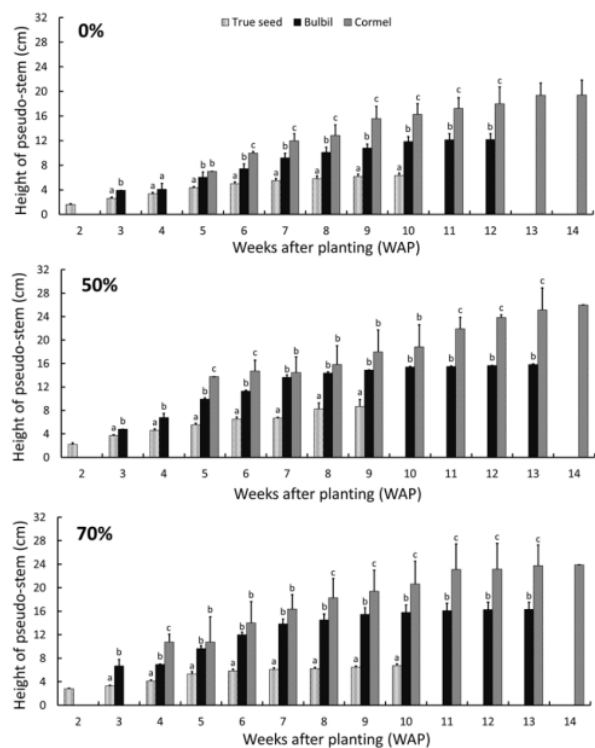


Figure 1 Height of pseudo-stem of konjac plant (*Amorphophallus muelleri*) grown using true seed, bulbil, or cormel under full sunlight (0%) exposure compared to 50 and 70% shading treatments.

Use of true seeds, bulbils, or cormels has been practiced in konjac cultivation has been known [3]. Fruit bunches of konjac consisted of seeds with various skin colors, i.e., green, yellow, and red. The skin colors associated with ripeness of the seed. Wardani *et al.* [18] reported that the red skin seeds grew better than those with yellow and green skins, as indicated by plant height, canopy diameter, and petiole diameter.

Santosa & Sugiyama [17] observed that growth and development of *A. paeoniifolius* were determined by planted cormel weight. Large cormel produced larger leaf and fresh biomass but decreased number of leaf. The lower yield obtained in sectioned large corm was related to the late shoot emergence, leading to shorter active vegetative period per growth cycle. Thus, whole seed corms of 100 to 200 g were recommended for konjac cultivation. Similar results were reported on bulbil as planting material. Plant height and plant diameter at an earlier growing phase significantly increased if larger of bulbil size was used. Larger bulbil size also significantly improved cormel diameter, thickness, and fresh weight [19].

Climatic conditions have been reported to affect growth in konjac plants. In this study, 50 and 70% shading treatments positively affected length of pseudo-stem (**Figure 1**), total number of leaflet (**Figure 2**), and other visual morphological characters (**Figure 3**) of konjac plants; yet the effects were not statistically significant (**Table 1**). Canopy of konjac plant actually consists of 1 compound leaf with 5 or more leaflets. Number of leaflets in the SOK plants were 5 and unchanged during life cycle of the 1st leaf. Number of leaflets in the 1st leaf of the BOK plant were 5 - 6 leaflets and of the COK plant were 6 - 9 leaflets.

Konjac plants exposed to shading rates of 50 and 70% exhibited higher net photosynthetic rate, lower transpiration rate, and increased yield than those exposed directly to sunlight [20]; 75% shading increased plant height, leaf number and leaf area [21]; 50% shade yielded 66% more corms [22]; and 75% shading fastened growth and the shaded plant reached commercial size of cormel in the 2nd year of cultivation [23].

Annual cycle of konjac plant follows intermittent active growth during rainy season and dormant during dry season. Mukherjee *et al.* [2] divided konjac plants into 2 groups relating to the climatic conditions. *A. paeonifolius* is a classic konjac plant Group-1 which has annual active-dormant cycle. Dormant cormels were stored during dry season and replanted at early rainy season. Konjac plant Group-2 has potential for all year around cultivation. This group was found in the deep equatorial zone which does not have a true dry period.

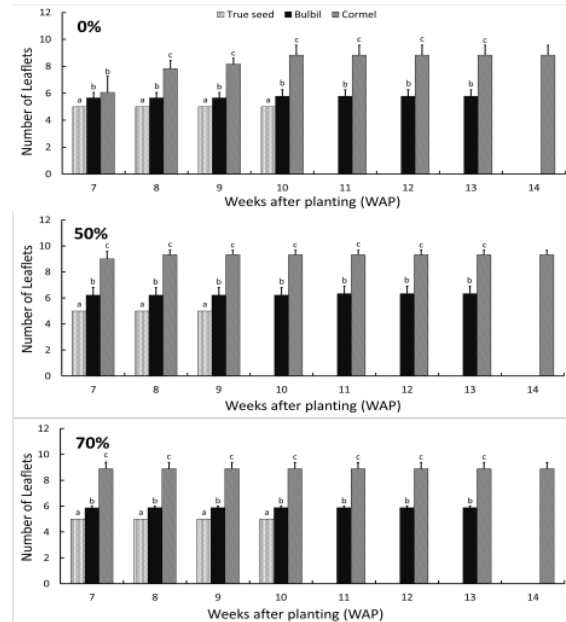


Figure 2 Number of leaflets in konjac plant (*Amorphophallus muelleri*) grown using true seeds, bulbils, or cormels under full sunlight (0 %) exposure compared to 50 and 70 % shading treatments.



Figure 3 Konjac plant (*Amorphophallus muelleri*) grown using true seeds, bulbils, and cormels (top to bottom rows) exposed to 0, 50 and 70 % (left to right column) at age 6 weeks after sowing.

Growth indicators and leaf succession

Relevant growth indicators for above-ground organs in konjac plant include height and diameter of pseudo-stem, number of leaflets, and total leaf area (**Table 2**) and for below-ground organs include diameter and thickness of cormel yield and length of roots (**Table 3**).

The SOK plant exhibited lower fresh weight, dry weights, and leaf water content in all above and underground organs than the BOK and COK plants. Lower value of growth parameters also observed in the BOK plants compared to the COK plant, except in pseudo-stem fresh and dry weights. Size-wise the SOK plant was smaller than that of BOK plant. Further, the BOK plants were also smaller than the COK plants. Similarity amongst all 3 plants grown using seed, bulbil, or cormel was only found in leaf water content (**Table 4**).

Table 2 Size differences in above-ground morphological characters of konjac (*Amorphophallus konjac*) planted using different planting materials.

Planting material	Pseudo-stem		Leaf	
	Length (cm)	Diameter (mm)	Number	Total area (cm ²)
True seed	11.69±1.13 a	4.68±0.51 a	7.70±0.18 a	118.2±23.13 a
Bulbil	25.66±2.96 b	8.47±0.98 b	12.06±1.35 b	351.9±57.51 b
Cormel	27.66±3.07 b	11.83±0.98 c	10.55±1.15 b	423.0±69.12 b

¹ Mean ± Standard error. Values followed by different letters within each column are significantly different at LSD.05

Table 3 Size differences in below-ground morphological characters of konjac (*Amorphophallus konjac*) planted using different planting materials.

Planting material	Cormel		Root length (cm)
	Diameter (mm)	Thickness (mm)	
True seed	17.47±1.74 a	14.80±1.06 a	13.76±0.80 a
Bulbil	24.38±3.35 b	19.29±2.05 b	16.61±1.22 b
Cormel	42.79±3.40 c	29.65±2.79 c	25.55±4.39 c

¹ Mean ± Standard error. Values followed by different letters within each column are significantly different at LSD.05

As in leaves of all plant species, eventually the young leaf of konjac plant will mature, senescence, and subsequently dies. The 1st leaf of the SOK died at 9 - 10 WAS; that of BOK died during 12 - 13 WAS; and that of the COK died after 14 WAS. The dead 1st leaf was immediately replaced by the 2nd leaf in each individual konjac plant. The 2nd leaf developed from the internal bud at pseudo-stem base of konjac plant [24].

Table 4 Fresh weight, dry weight, and water content in konjac (*Amorphophallus konjac*) planted using different planting materials.

Planting material	Fresh weight (g)	Dry weight (g)	Water content (%)
<i>Pseudo-stem</i>			
True seed	2.66±0.70 a	0.20±0.05 a	91.61±1.15 a
Bulbil	16.04±4.22 b	1.14±0.45 b	93.93±0.54 b
Cormel	25.28±5.85 b	1.40±0.24 b	94.03±0.49 b
<i>Cormel</i>			
True seed	4.57±1.04 a	0.96±0.19 a	76.02±2.31 a
Bulbil	12.62±3.36 b	2.11±0.53 b	81.36±1.24 b
Cormel	44.48±5.41 c	6.93±0.83 c	84.05±0.63 c
<i>Roots</i>			
True seed	5.27±1.40 a	0.54±0.14 a	88.68±0.95 a
Bulbil	9.36±2.21 b	0.74±0.17 a	89.98±1.39 a
Cormel	31.65±7.07 c	2.55±0.52 b	90.88±1.62 a

Mean ± Standard error. Values followed by different letters within each column are significantly different at LSD.05

Increase in height and diameter of pseudo-stem, number of leaflets, length of the main midrib, and leaflet width from the 1st to the 2nd leaf can be visually compared amongst the SOK, BOK, and COK plants (Figure 4). There was no significant change in all growth parameters between the 1st and 2nd leaf in the SOK plants. However, increase in height and diameter of pseudo-stem between the 1st and 2nd leaf were observed in both BOK and COK plants. However, only the BOK plants exhibited increase in number of leaflets and midrib length. None of the SOK, BOK, and COK plants were significantly increased their leaf width. In general, based on growth parameters measured in the 2nd leaf, plant grown using cormel performed better than those grown using true seed and bulbil.

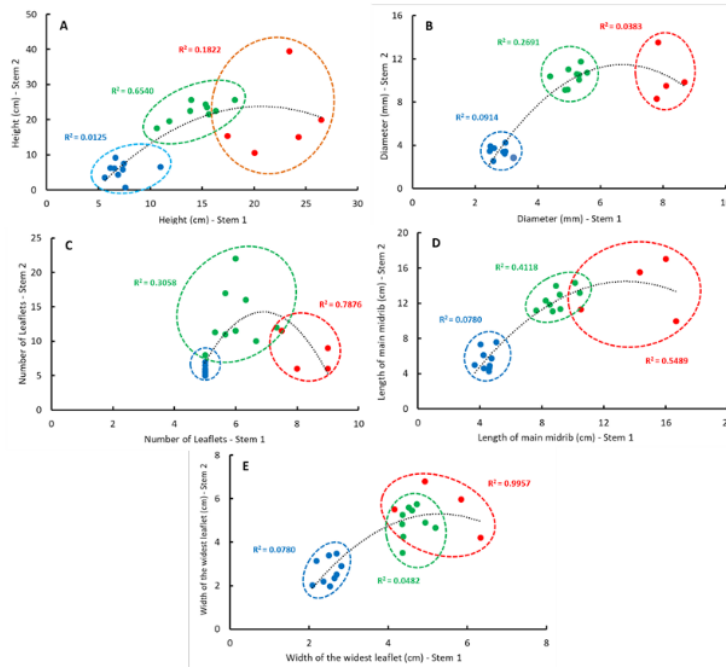


Figure 4 Visual comparison of morphological characters in the first 2-leaf cycle of konjac grown using true seed (blue), bulbil (green), or cormel (red) as planting material.

Significance of leaf and leaf area estimation models

Leaf area (LA) is a very significant morphological trait in konjac plant since the above ground vegetative organs of this unique plant only consisted of a single pseudo-stem and compound leaf. Stem-like organ in konjac functions similar to pseudo-stem in banana but formed from layers of leaf sheath [25]; therefore, it should be properly classified as pseudo-stem. Botanically, this pseudo-stem is a petiole [24]. Konjac plant may have 1 or more pseudo-stems which grows directly from the sown planting materials. At upper end of the pseudo-stem of konjac, it splits into 3 short petiolules of the compound leaf. Each compound leaf consists of 5 or more leaflets (**Figure 3**).

Number of leaflets may increase during leaf development stage. There is also a straight midrib in each leaflet from the point of attachment to the tip of the leaflet blade. Commonly, the leaflet blade is not symmetrically divided by its midrib. One half has a distinctive elliptic shape, but the other half of the leaflet blade merges with the blade of the main leaf. Architecture of konjac leaf and canopy becomes more complicated as the plant get larger and older. This complicated leaf structure makes it more challenging in developing leaf area estimation in konjac plant.

The non-destructive measurement of leaf area is very useful in studying konjac plant due to limited available above-ground organ. The LA estimation model can be developed using the best-fit regression models with simple allometric measurements [26-28]. This study used the average of all leaflet length (L_L) or average of all leaflet width (W_L) as a single predictor (**Figure 5**). A more comprehensive and specific predictor for konjac leaf, i.e., the total midrib length of all leaflets (TL_M) or the multiplication product of total midrib length of all leaflets and average of all leaflet width ($TL_M \times AW_L$) could increase accuracy of LA estimation using the 0-intercept linear model (**Figure 6**).

Leaf of konjac plant consists of 5 or more leaflets with similar shape but may vary on their sizes. This condition opens opportunity to develop LA estimation model using average length and width of all leaflets as predictors. Each regression has its own trend line as shown in the 3 LA estimation models in **Figure 5**. In this specific cases, i.e., leaf with 5 or more leaflets, the exponential model fit nicely to distribution of leaflet length or width data. However, if the $TL_M \times AW_L$ predictor used, as in **Figure 6**, the 0-intercept linear model exhibited the best performance in estimating LA in konjac plant ($R^2 > 0.99$). Meanwhile, if only TL_M was used as predictor, accuracy of LA estimation model notably decreased ($R^2 < 0.94$).

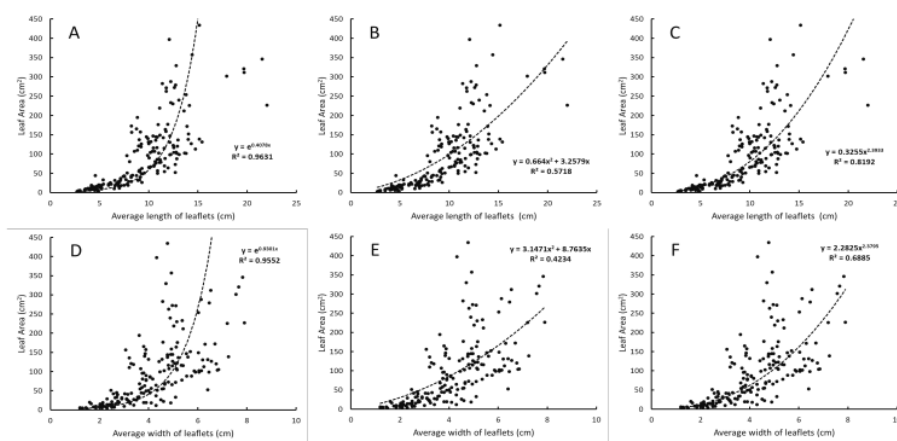


Figure 5 Average leaflet length (A-C) and width (D-F) as predictors for estimating area of *Amorphophallus konjac* compound leaf using exponential, 0-intercept quadratic, or power regression.

Benefits of developing leaf area estimation models are for successively measuring the same individual leaf since the process is non-destructive and accurately estimating leaf area without using sophisticated and expensive instruments [29]. Most of available models, however, did not use geometrical consideration in developing the models [30-32]. Commonly used predictors for estimating leaf area (LA) are leaf length (L_L), leaf width (W_L), or multiplication product of length and width ($L_L \times W_L$). Geometrically, if $L_L = 0$ or $W_L = 0$ then LA has to be 0. For this reason, 0-intercept regression models

were recommended for LA estimation in the previous studies [29-32], including the oldest model of Lakitan [30].

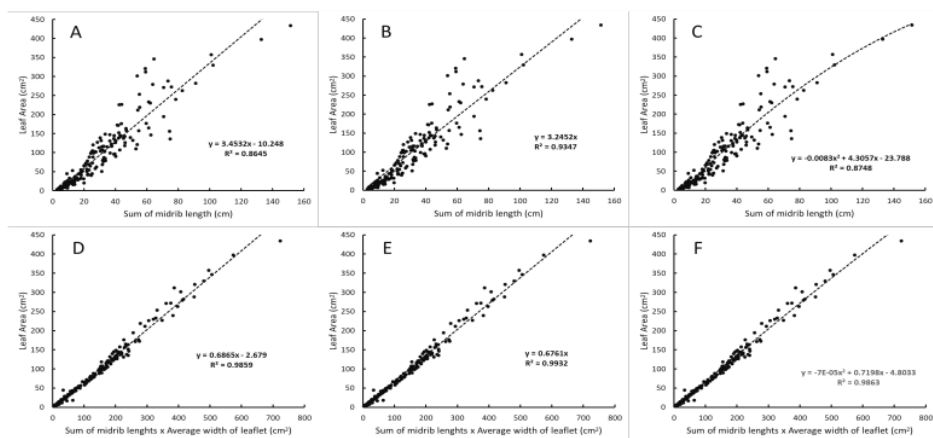


Figure 6 Sum of midrib length (A-C) as single or combined with average width of leaflet (D-F) as double predictors for estimating area of *Amorphophallus konjac* compound leaf using linear, 0-intercept linear, or quadratic regression.

Two regression models are nicely and consistently fit with the trend of relationship between L_L or W_L and LA in single blade leaf, i.e., power regression and quadratic regression. If $L_L \times W_L$ is used as predictor, the best fit model is the linear regression. For compound leaf with multiple leaflets, the exponential regression exhibited better R^2 value than either power or quadratic regression (Figure 5). However, the best and consistent model for predicting LA was the 0-intercept linear regression using the multiplication product of the sum of midrib lengths and average width of leaflets as predictor (Figure 6). This is a genuine and recommended approach in estimating LA with this selected predictor.

Conclusions

It takes much longer time for shoot emergence if the cormel is used as a planting material, yet it takes longer time to harvest if the true seed is used. Use of bulbils seems to be the best option as planting material in konjac cultivation since the bulbil emerge much earlier than the cormel and grow almost as fast as cormel does. Shading at 50 and 70 % did not significantly affect shoot emergence and growth. The recommended leaf area estimation model is $LA = 0.6761 (TL_M \times AW_L)$. Further research on effects of hydro-priming and osmo-priming using various beneficial chemicals or growth hormones are needed to accelerate emergence and effectively break dormancy in konjac plants. Other avenues to explore for future research in konjac plants include establishing continuous cultivation based on water management system and use of 'Group 2' konjac species.

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