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# GROWTH PERFORMANCE AND PRODUCTION OF *Limnocharis flava* (L.) BUCHENAU FOR VEGETABLE CROP

[1]S. Noorasmah, [2]Z. Muta Harah, [2]B. Japar Sidik and [2]A. Aziz

[1]Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia.
[2]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia.

## ABSTRACT

*Limnocharis flava (L.)* Buchenau is a fast growing aquatic plant often associated with rice fields and drainage systems. When present in abundant, it is a serious weed, often competing for nutrients and space. In the region of Sarawak, Malaysia, plants are harvested from the wild and offered for sale in native markets as edible vegetable and consumed among local urban peoples. There has been no attempt to propagate the plants through cultivation. Hence, a study was conducted to evaluate the growth performance of *L. flava* toward water nutrient uptake and plant production. *Limnocharis flava* can be propagated from seeds or plantlets in created environment, e.g., in tank. Plants propagated from seeds showed higher increased in plant vegetative parameters, i.e., plants' height, number of leaf, blade length and width, petiole diameter, and inflorescence compared to plants propagated from plantlets. Comparing growth performance of *L. flava* and culture water nutrients based on multivariate non-parametric procedure BV-STEP, increased in number of inflorescence from plant propagated from seeds was moderately correlated with $NO_3^-$, while increased in blade length in plants propagated from plantlets were related to a combination of nitrogen sources $NO_2^-$, $NO_3^-$and $NH_3^-$. Seven harvestings performed at two weeks interval after five weeks of transplanting showed the yield of *L. flava* shoots from seeds propagation was comparatively higher than those propagated from plantlets.

**Keywords:** Growth performance; *Limnocharis flava*; nutrient uptake; tank culture; yield.

ISSN: 2455-6939

Volume:02, Issue:02

## INTRODUCTION

Plants are able to reproduce by seeds and asexually by means of vegetative organs. Plants from habitat that are unfavorable for seedling establishment tend to rely largely on vegetative reproduction (Sculthorpe, 1985; Fenner, 1985; Fenner and Thompson, 2005). Asexual reproduction is the dominant form of reproduction for aquatic plants, i.e., shoot fragments (*Ceratophyllum*), turions (*Utricularia*), inflorescence plantlets (*Echinodorus*), runners or stolons (*Cryptocoryne*), rhizomes (*Typha*), stem tubers (*Sagittaria*), root tubers (*Nymphoides*) and corm (*Aponogeton*) (Sculthorpe, 1985; Cronk and Fennessy, 2001). *Limnocharisflava*can expand its population either by seeds. A fruit of *L. flava* contains numerous seeds, e.g., 1000 seeds (Kotalawala *et al.,* 1976), 470 to 640 (Quan, 2000) and 524 to 1547 (Brooks et al., 2008). Besides increasing its population through the seeds, the plant also propagates vegetatively through plantlets or bulbils formed at the apex of flower stalks (Wilder, 1974; Nayar and Sworupanandan, 1978; Quan, 2000). Studies have been conducted on the general aspects of *L. flava* such as ontogeny and anatomy of the flower (Kaul, 1967), plant development (Wilder, 1974), germination and establishment of the seedlings (Kaul, 1978), fruit and mechanism of seed dispersal (Nayar and Sworupanandan, 1978), propagation and seed dispersal (Kostermans et al*.,* 1987), agronomy (van den Bergh, 1994), breeding systems (Quan, 2000), seed production and maturation (Brooks et al*.,* 2008). Abhilash et al*.* (2008) noted on infestation by *L. flava* of rice fields in Southern India through vigorous reproduction of plantlets. Crop production aspects of this plant are few and reported by van den Bergh (1994) and (Maisuthisakul et al., 2008). According to van den Bergh (1994), *L. flava* are cultivated in fertile soil and harvested after 2 to 3 months and widely practiced in West Java and Thailand. In region such as Sarawak, *Limnochari*s plants have not been cultivated and they are gathered from wild and offered for sale as a leafy vegetable in local markets (Voon et al., 1988; Saupi et al., 2009). This present study was conducted to propagate the plant and evaluate the growth performance and yield using seeds and plantlets in created environment using fiberglass tank.

## MATERIALS AND METHODS

### Plant Materials

Propagation of *L. flava* was conducted from 16$^{th}$ of August to 27$^{th}$ of December 2010 (19 weeks) at Ladang Kongsi, Taman Pertanian Universiti Universiti Putra Malaysia Bintulu Sarawak Campus. The experiment was design in completely randomized design (CRD) for 30 seedlings and 30 plantlets. Seeds and plantlets collected from waterway at Public Library Mukah Sarawak, Malaysia (N 02° 54.283' and E 112° 06.183') were first germinated to obtain the seedlings. Plantlet is the rooted vegetative plant that developed from the centre of inflorescence. Both plant materials were planted into sediments composed of 3 top soil:2 sand:1 compost by weight in 240

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Volume:02, Issue:02

cm x 120 cm x 50 cm fiber glass tank (Figure 1) and with planting distance of 30 cm x 30 cm. The planting of *L. flava* distance and depth of water practiced in this experiment followed the growing condition of this plant in flooded rice field at West Java, Indonesia as reported by van den Bergh (1994).

**The Growth Performance**

The plant performance in this study involve a measure of some suitable character; leaf length, leaf width or a ratio of the two as these were related to leaf area (for measurement available for photosynthesis) and, flower number reflecting the reproductive capacity of an individual (Kershaw, 1964). Other than those parameters mentioned above, height of plant, leaf petiole length and diameter, number of leaf inflorescence formed were recorded weekly for six weeks started from August 16$^{th}$ to September 27$^{th}$ in 2010 to determine the growth performance (Figure 2).

Water samples were collected from culture tanks at the initial of planting and thereafter weekly for determination of the concentration of nitrate ($NO_3^-$) and nitrite ($NO_2^-$), ammonium ($NH_3^-$) and ortho-phosphate ($PO_4^{3-}$).The water samples were filtered by using Rocker® 600 filter vacuum set with Whatman filter paper No.1, Whatman glass microfiber filter GF/C and Whatman cellulose nitrate membrane filter 0.45 µm following the method of EPA (2009) prior to nutrient analyses. The concentrations of nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonia ($NH_3^-$) and orthophosphate ($PO_4^{3-}$) were determined using a portable spectrophotometer-DR/2400 (Hach DR/2400 Spectrophotometer Procedure Manual 2002).

**Yield**

The tender edible shoot of *L. flava* comprises leaves, (matured and young) and inflorescence clusters were harvested from October 4$^{th}$ until December 27$^{th}$ in 2010. Harvested components were cleaned under running water and residual moisture evaporated at room temperature. The fresh weight, diameter and length of shoot were recorded from plants propagated by seed and plantlet. Seven similar harvestings were conducted at two weeks interval until the yield production declined. Two hundred gram of fertilizer (Garden Well 45® of 15% N: 15% P: 15% K) per tank was applied after 4$^{th}$ harvesting to maintain the growth performance.

**Statistical Analysis**

The growth performances; i.e., increased in plant height, petiole diameter, blade length, blade width, number of leaf, number of inflorescence and the mean values of the dimension and yields

ISSN: 2455-6939

Volume:02, Issue:02

of harvested shoots of *L. flava* propagated from seeds and plantlets were compared using SAS V 9.2 with single factor AVOVA ($p<0.05$) and t-test ($p<0.05$).The growth performance parameters response and nutrient contents of culture water during the cultivation were also compared by using Plymouth Routines in the Multivariate Ecological Research (PRIMER) statistical software package version 5 (Clarke and Warwick, 2001). The similarity matrix of the growth performance parameters was classified according to Bray-Curtis similarity (Bray and Curtis, 1957) by hierarchical agglomerative clustering via complete linkage method and followed by multivariate non-parametric procedure BV-STEP to verify the nutrient or nutrients in the culture water that may explain the increased in growth performance parameters in the experiments.

## RESULTS AND DISCUSSION

### Growth Performance

The growth performance of *L. flava* from seeds and plantlets are presented in Figure 3. Plant consistently produced leaves (Figure 3a) and inflorescences first appearance two weeks after transplanting (Figure 3b). The increased in plant height (Figure 3c), blade length (Figure 3e) and width (Figure 3f) did not showed obvious difference in the early growth phase. At week 4 after transplanting, plants propagated from seed showed increased in height than plants propagated from plantlet and later both attained similar increased in height after week 5 after transplanting. The increased in petiole diameter of propagated plants from seeds showed significantly higher at week 3 to week 6 after transplanting compared to the plants propagated from plantlets (Figure 4d).

The growth performance responses, i.e., increased in number of leaf and inflorescence, plant height, petiole diameter, blade length and blade width (Figure 3) were compared with culture water nutrient; $NO_3^-$, $NO_2^-$, $NH_3^-$ and $PO_4^{3-}$ (Table 1) by using multivariate non-parametric procedure BV-STEP. The spearman correlation, *p* value between the variables represents the relationship, where *p* value up to 0.33 is considered weak relationship, between 0.34 to 0.66 medium strength relationship and over 0.67 as strong relationship. Based on multivariate non-paramateric procedure BV-STEP, the likely nutrient or combination of nutrients involved in producing positive response to the growth performance parameters is shown in Table 2. *Limnocharis flava* may not selectively absorbed one nutrient at a time, but the uptake may involve a combination of absorbable nutrient in the culture water. The growth performance from plant propagated from seeds as indicated by increased in number of leaf, plant height, petiole diameter, blade length and blade width were having weak correlation, *p* value 0.046 to 0.272 with the nutrient constitutions, while moderate correlation *p* value 0.474 explained the increased number of florescence was related to $NO_3^-$ in culture water. Increased in blade length of plants

ISSN: 2455-6939

Volume:02, Issue:02

propagated from plantlets showed moderate correlation $p$ value 0.414 with nitrogen sources of $NO_2^-$, $NO_3^-$, and $NH_3^-$. Ruess et al. (1983) and Ozimek et al. (1993) also reported $NO_3^-$ was increased the plant biomass of other aquatic plants, i.e., *Kyllingi nervosa* Steud. and *Elodea* respectively.

**Yield**

Seven harvesting activities were performed after five weeks of transplanting plants from polybag to tank culture to investigate the production of *L. flava* in 2.88 $m^2$ area at two weeks interval (Figure 4). The yield of shoots increased in 1st to 3rd harvesting and declined in 4th due to the infestation of yellow leg aphids (*Aphis gossypii* Glover, Homoptera: Aphididae) and young leaf soft rot disease. The yield of shoots dramatically increased after applying insecticide foliar spray. The yield of shoot from seed propagation was significantly higher ($p<0.05$) than propagated from plantlets. Dimension analyses of harvested shoots from plants propagated from seeds and plantlets are summarized in Tables 3 and 4. There was no discernible difference in the dimension of shoots harvested from both propagated plants in culture. The cultured *L. flava* shoot lengths were in the range of marketed *L.flava*'s shoots which were collected from the wild (Table 3).

In this study, *L. flava* was grown in tank system for five months cropping season. The comparison of production of cultivated *L. flava* plants in tanks with other production from various studies (van den Bergh, 1994) is shown in Table 5. This study showed that plantlet can be used for the cultivation of *L. flava.* In West Java, Indonesia, propagation by plantlet is commonly used in paddy fields as reported by van den Bergh (1994). The yield of *L. flava* in this cultivation was lower than in rice field cultivation as reported by van den Bergh (1994). This is partly attributed to the infestation of *A. gossypii* which decreased the yield of *L. flava* cultivated in tanks.

**CONCLUSION**

*Limnocharis flava* can be propagated from seeds and plantlets in created environment using tank. This species was cultured with five months cropping season. The increased in height of plant, petiole and blade size did not showed obvious difference in the early growth but at week 4 the plant propagated from seed was comparatively higher in the increment in height than plants from plantlet. Based on weekly monitoring on water nutrients, the plant selectively absorbed more than one nutrient available in the water sources. There is no specific nutrient that is responsible for the growth performance parameters. In the production study, the yield of shoots from seed propagation was comparatively higher than those from plantlet. The yield of *L. flava* in this present study was lower than those obtained in rice field cultivation in Indonesia.

ISSN: 2455-6939

Volume:02, Issue:02

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ISSN: 2455-6939

Volume:02, Issue:02

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ISSN: 2455-6939

Volume:02, Issue:02

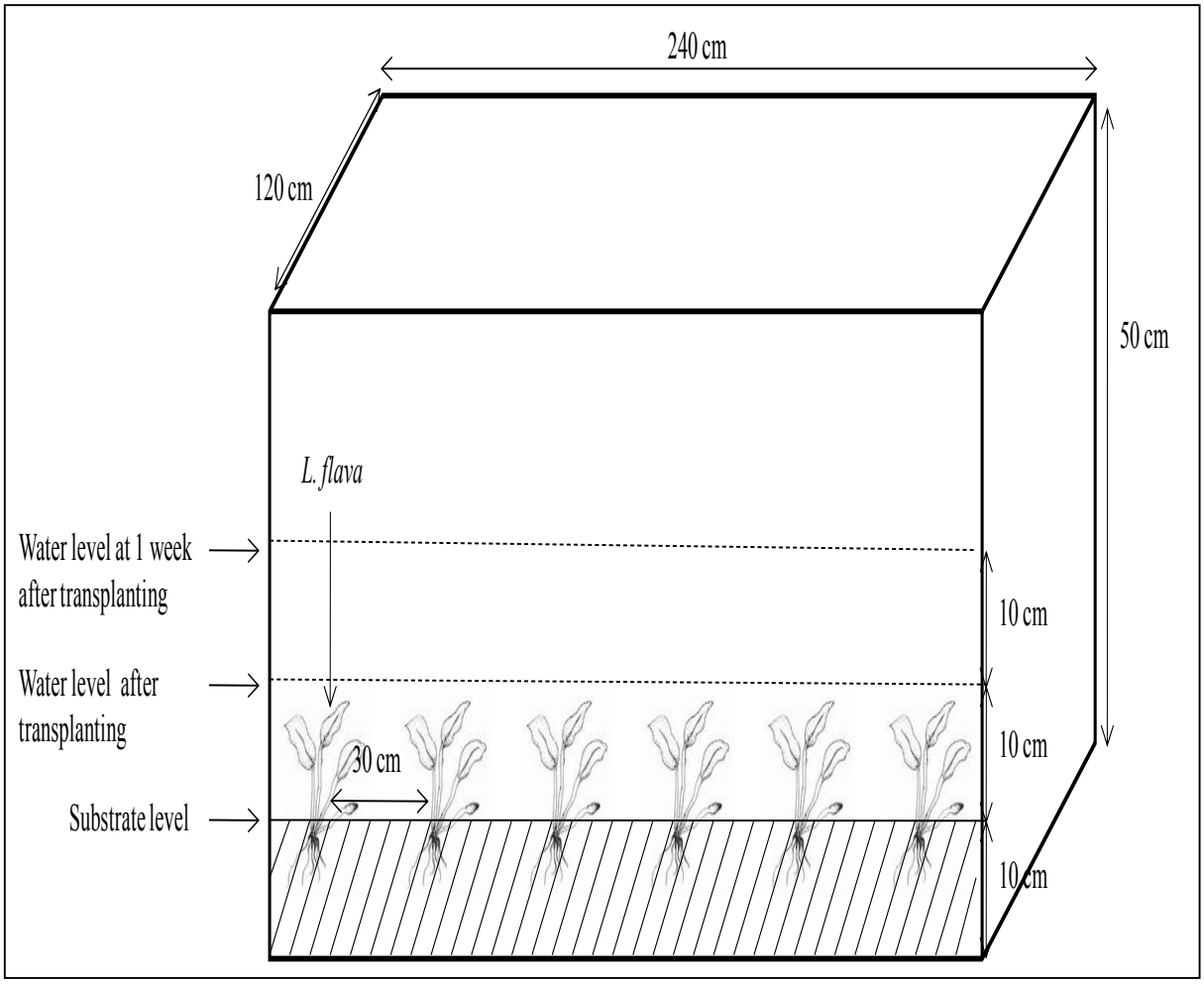


**Figure 1: The plants (seedlings) propagated from seeds or plantlets were grown in tank at the distance of 30 cm x 30 cm in 10 cm substrate level and flooded with aged tap water at 10 cm level.**

ISSN: 2455-6939

Volume:02, Issue:02

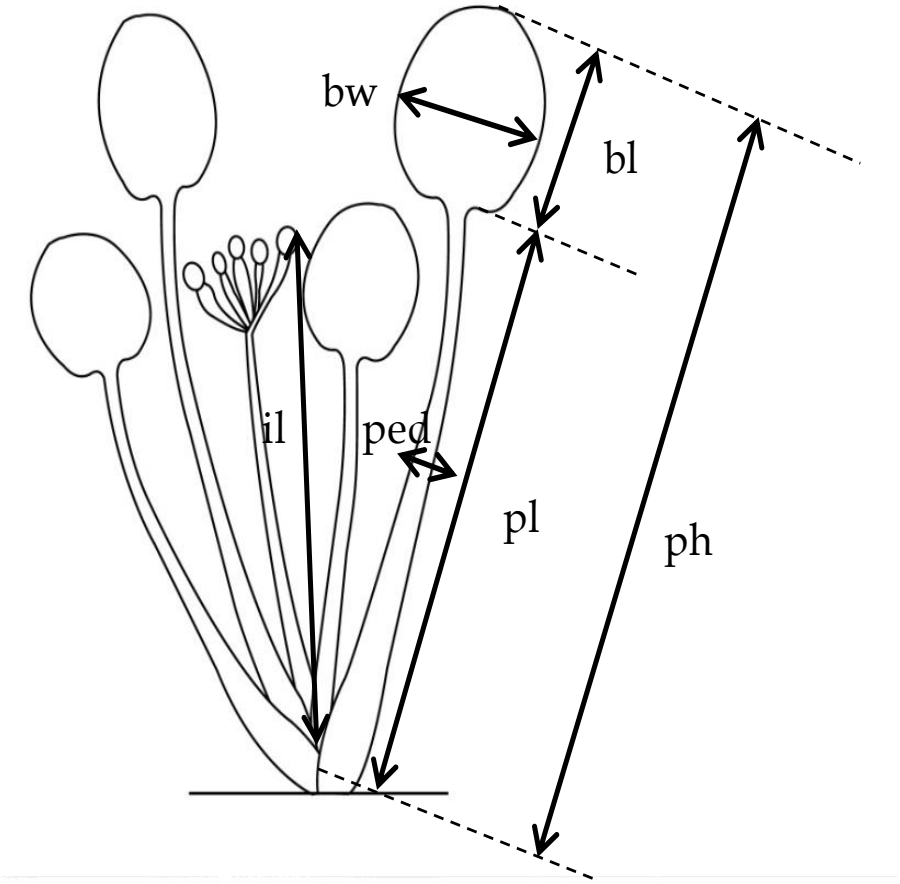


**Figure 2: The illustration of measurement taken for recording various components of *L. flava* - plant height (ph), blade length (bl), blade width (bw), petiole length (pl), petiole diameter (ped) and inflorescence length (il).**

ISSN: 2455-6939

Volume:02, Issue:02

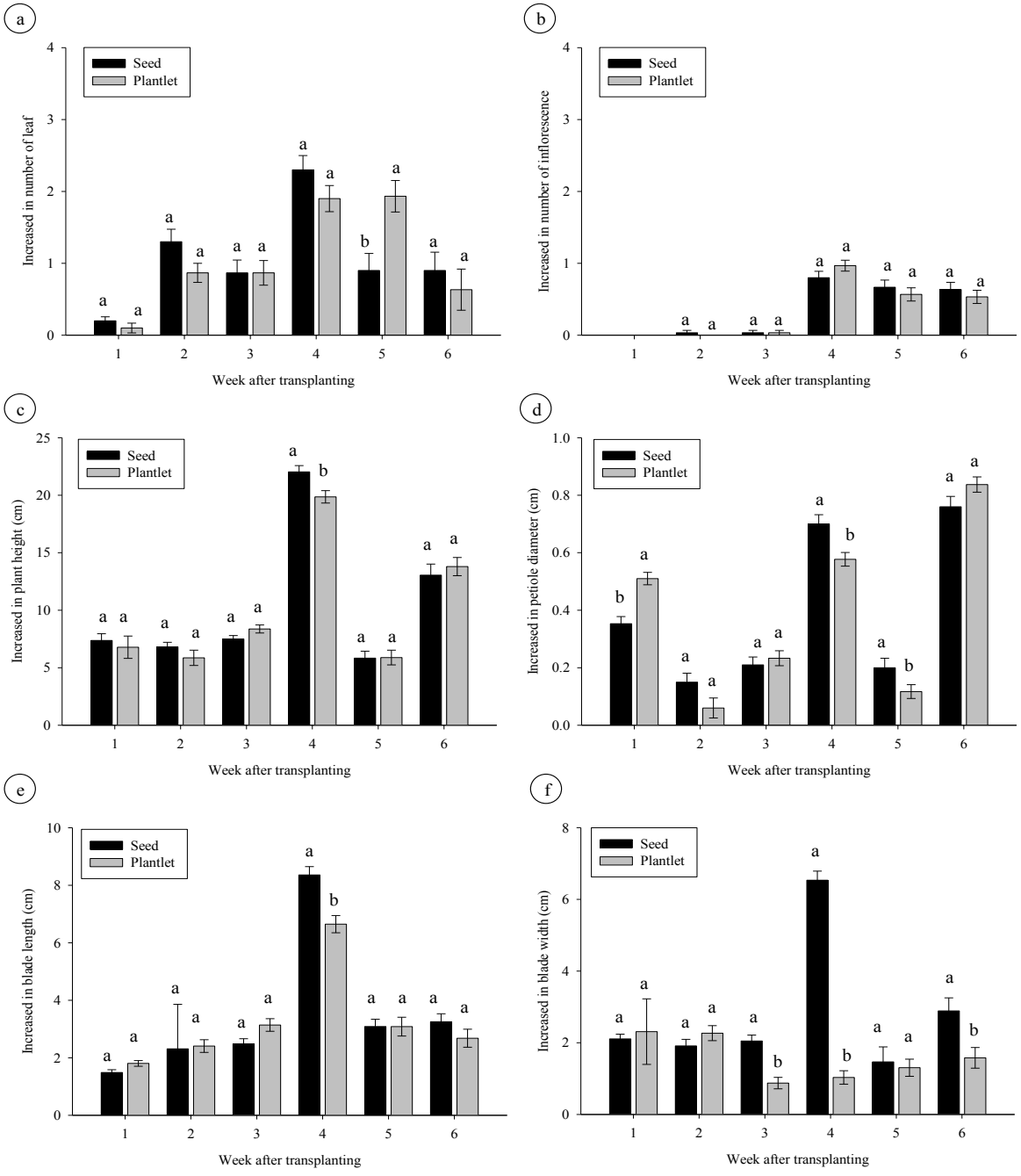


**Figure 3: The growth performance of *L. flava* from seeds and plantlets. (a) Increased in number of leaf, (b) increased in number of inflorescence, (c) increased in plant height, (d) increased in petiole diameter, (e) increased in blade length and (f) increased in blade width (given as means ± s.e, n=30). The bar sharing a common letter at the**

ISSN: 2455-6939

Volume:02, Issue:02

**same weeks are not statistically significant between planting materials (seeds and plantlets) according to t-Test ($p$<0.05), i.e., a > b.**

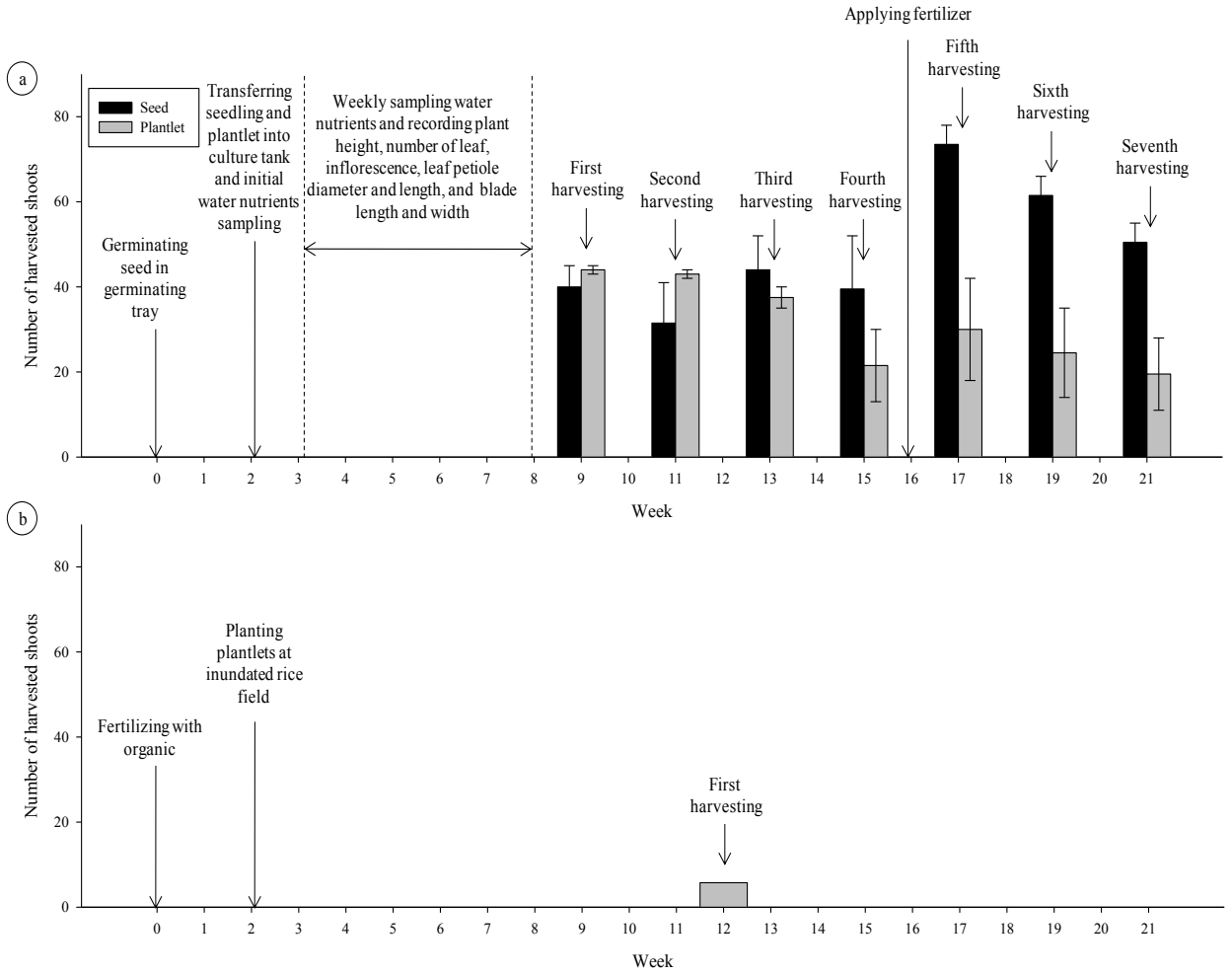


**Figure 4: Comparison of yield of *L. flava* two different cultured systems in 2.88 m$^2$ area. (a) Number of harvested shoot harvested from tank culture of present study, i.e., all values are given as means ± s.e and (b) number of harvested shoots from inundated rice field as reported by van den Bergh (1994).**

ISSN: 2455-6939

Volume:02, Issue:02

**Table 1: Nutrient content in culture water of *L. flava* propagated from seeds and plantlets.**

| Week | $NH_3^-$ (ppm) | | $NO_2^-$ (ppm) | | $NO_3^-$ (ppm) | | $PO_4^{3-}$ (ppm) | |
|---|---|---|---|---|---|---|---|---|
| | Plant material | | Plant material | | Plant material | | Plant material | |
| | Seed | Plantlet | Seed | Plantlet | Seed | Plantlet | Seed | Plantlet |
| 1 | $0.052 \pm 0.004^b$ | $0.078 \pm 0.004^a$ | $0.025 \pm 0.009^b$ | $0.057 \pm 0.009^a$ | $1.450 \pm 0.081^b$ | $1.533 \pm 0.055^a$ | $0.427 \pm 0.029^{n.s}$ | $0.518 \pm 0.032^a$ |
| 2 | $0.052 \pm 0.005^b$ | $0.080 \pm 0.001^a$ | $0.018 \pm 0.003^b$ | $0.050 \pm 0.007^a$ | $1.350 \pm 0.050^b$ | $1.550 \pm 0.022^a$ | $0.418 \pm 0.029^{n.s}$ | $0.473 \pm 0.024^{n.s}$ |
| 3 | $0.052 \pm 0.004^b$ | $0.070 \pm 0.001^a$ | $0.016 \pm 0.002^b$ | $0.059 \pm 0.009^a$ | $1.417^b \pm 0.040$ | $1.533 \pm 0.033^a$ | $0.395 \pm 0.043^{n.s}$ | $0.482 \pm 0.011^{n.s}$ |
| 4 | $0.050 \pm 0.003^b$ | $0.070 \pm 0.004^a$ | $0.010 \pm 0.001^b$ | $0.057 \pm 0.009^a$ | $1.350 \pm 0.043^b$ | $1.433 \pm 0.021^a$ | $0.270 \pm 0.036^b$ | $0.472 \pm 0.011^a$ |
| 5 | $0.055 \pm 0.003^{n.s}$ | $0.057 \pm 0.003^{n.s}$ | $0.009 \pm 0.001^b$ | $0.054 \pm 0.008^a$ | $1.333 \pm 0.042^b$ | $1.417 \pm 0.070^a$ | $0.215 \pm 0.026^b$ | $0.410^a \pm 0.016$ |
| 6 | $0.050 \pm 0.004^{n.s}$ | $0.057 \pm 0.002^{n.s}$ | $0.008 \pm 0.001^b$ | $0.052 \pm 0.009^a$ | $1.267 \pm 0.033^b$ | $1.583 \pm 0.065^a$ | $0.157 \pm 0.011^b$ | $0.382 \pm 0.014^a$ |

All values are given as mean ± s.e, n=6. Different superscript alphabets in the same column of nutrients within same week indicate significant difference at $p<0.05$ (ANOVA, t-Test), i.e., a > b; n.s is not significantly different.

ISSN: 2455-6939

Volume:02, Issue:02

**Table 2: The drivers, i.e., likely nutrient or nutrients combination related to the observed growth performance variables in *L. flava* throughout the growth experiments based on multivariate non-parametric procedure BV-STEP.**

| Parameters | Plant material | Number of nutrient | Spearman rank correlation (*p*) | Best nutrient combination |
|---|---|---|---|---|
| Increased in number of leaf | Seed | 1 | 0.272 | $NO_3^-$ |
| | Plantlet | 2 | 0.213 | $NH_3^-$, $PO_4^{3-}$ |
| Increased in number of inflorescence | Seed | 1 | 0.474 | $NO_3^-$ |
| | Plantlet | 2 | 0.093 | $NH_3^-$, $PO_4^{3-}$ |
| Increased in plant height | Seed | 4 | 0.146 | $NH_3^-$, $NO_3^-$, $NO_2^-$, $PO_4^{3-}$ |
| | Plantlet | - | 0.000 | No trend was obtained |
| Increased in petiole diameter | Seed | 4 | 0.046 | $NH_3^-$, $NO_3^-$, $NO_2^-$, $PO_4^{3-}$ |
| | Plantlet | 3 | -0.020 | $NO_3^-$, $NO_2^-$, $PO_4^{3-}$ |
| Increased in blade length | Seed | 3 | 0.157 | $NH_3^-$, $NO_3^-$, $NO_2^-$ |
| | Plantlet | 3 | 0.414 | $NH_3^-$, $NO_3^-$, $NO_2^-$ |
| Increased in blade width | Seed | 4 | 0.136 | $NH_3^-$, $NO_3^-$, $NO_2^-$, $PO_4^{3-}$ |
| | Plantlet | 1 | 0.285 | $NO_3^-$ |

ISSN: 2455-6939

Volume:02, Issue:02

**Table 3: Shoot length and inflorescence length of *L. flava* propagated from seeds and plantlets.**

| Harvesting time | Young leaf length (cm) | | Rolled leaf length (cm) | | Inflorescence length (cm) | |
|---|---|---|---|---|---|---|
| | Plant material | | Plant material | | Plant material | |
| | Seed | Plantlet | Seed | Plantlet | Seed | Plantlet |
| 1 | $83.23\pm1.25^{b}$ (n=16) | $86.90\pm1.14^{a}$ (n=23) | $40.00\pm3.62^{n.s}$ (n=38) | $39.76\pm3.28^{n.s}$ (n=39) | $32.91\pm4.50^{n.s}$ (n=27) | $41.68\pm3.82^{n.s}$ (n=26) |
| 2 | $67.33\pm3.12^{b}$ (n=16) | $75.22\pm2.25^{a}$ (n=26) | $31.86\pm3.77^{b}$ (n=23) | $36.25\pm2.99^{a}$ (n=34) | $35.58\pm3.26^{n.s}$ (n=24) | $37.85\pm3.63^{n.s}$ (n=26) |
| 3 | $65.72\pm2.94^{n.s}$ (n=22) | $70.78\pm2.08^{n.s}$ (n=21) | $36.23\pm2.79^{n.s}$ (n=36) | $37.11\pm3.09^{n.s}$ (n=29) | $29.99\pm3.10^{n.s}$ (n=30) | $32.56\pm2.56^{n.s}$ (n=25) |
| 4 | $63.16\pm2.31^{a}$ (n=19) | $54.45\pm2.76^{b}$ (n=10) | $33.31\pm2.30^{n.s}$ (n=34) | $33.09\pm3.51^{n.s}$ (n=22) | $33.89\pm2.62^{n.s}$ (n=24) | $26.21\pm5.06^{n.s}$ (n=11) |
| 5 | $79.03\pm1.20^{a}$ (n=36) | $69.62\pm2.07^{b}$ (n=13) | $43.34\pm2.62^{a}$ (n=74) | $37.79\pm3.43^{b}$ (n=31) | $37.51\pm3.02^{n.s}$ (n=37) | $39.97\pm4.14^{n.s}$ (n=19) |
| 6 | $76.94\pm0.91^{a}$ (n=28) | $69.58\pm1.10^{b}$ (n=10) | $44.22\pm2.69^{a}$ (n=63) | $36.65\pm3.55^{b}$ (n=25) | $38.59\pm3.16^{n.s}$ (n=32) | $37.66\pm4.68^{n.s}$ (n=14) |
| 7 | $74.36\pm0.92^{a}$ (n=23) | $67.80\pm1.61^{b}$ (n=8) | $42.35\pm2.83^{a}$ (n=52) | $32.41\pm3.65^{b}$ (n=21) | $33.70\pm3.65^{n.s}$ (n=26) | $27.64\pm5.57^{n.s}$ (n=10) |
| Marketed *L. flava*'s shoot* | 30.18 – 59.20 | | 27.57 – 65.14 | | 25.33 – 54.05 | |

All values are given as mean ± s.e. Different superscript alphabets in the same column of shoots dimensions within same harvesting week indicate significant difference at $p<0.05$ (ANOVA, t-Test), i.e., a > b; n.s is not significantly different. *The range of shoot length of marketable wild *L. flava* was used to compare the cultured shoots.

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Volume:02, Issue:02

**Table 4: Shoot weight and inflorescence weight of *L. flava* propagated from seeds and plantlets.**

| Harvesting time | Young leaf weight (g) | | Rolled leaf weight | | Inflorescence weight (g) | |
|---|---|---|---|---|---|---|
| | Plant material | | Plant material | | Plant material | |
| | Seed | Plantlet | Seed | Plantlet | Seed | Plantlet |
| 1 | $51.81\pm1.55^{n.s}$ (n=16) | $53.91\pm1.38^{n.s}$ (n=23) | $18.32\pm2.52^{n.s}$ (n=38) | $18.67\pm2.60^{n.s}$ (n=39) | $12.37\pm2.04^{n.s}$ (n=27) | $15.62\pm1.87^{n.s}$ nN=26) |
| 2 | $34.44\pm2.91^{b}$ (n=16) | $43.88\pm3.01^{a}$ (n=26) | $13.00\pm2.36^{n.s}$ (n=23) | $17.44\pm2.42^{n.s}$ (n=34) | $9.00\pm1.11^{b}$ (n=24) | $12.65\pm1.44^{a}$ (n=26) |
| 3 | $32.14\pm2.73^{b}$ (n=22) | $39.71\pm2.34^{a}$ (n=21) | $13.17\pm1.59^{n.s}$ (n=36) | $15.17\pm2.24^{n.s}$ (n=29) | $7.63\pm1.08^{n.s}$ (n=30) | $8.52\pm0.95^{n.s}$ (n=25) |
| 4 | $28.16\pm2.33^{n.s}$ (n=19) | $24.80 \pm 1.74^{n.s}$ (n=10) | $11.88\pm1.44^{n.s}$ (n=34) | $12.41\pm1.96^{n.s}$ (n=22) | $9.13\pm1.08^{n.s}$ (n=24) | $6.45\pm1.66^{n.s}$ (n=11) |
| 5 | $40.94\pm2.04^{a}$ (n=36) | $33.46\pm2.55^{b}$ (n=13) | $17.80\pm1.63^{n.s}$ (n=74) | $15.10\pm1.6^{n.s}$ (n=31) | $10.68\pm1.16^{n.s}$ (n=37) | $11.56\pm1.67^{n.s}$ (n=19) |
| 6 | $37.00\pm1.69^{a}$ (n=28) | $30.80\pm1.88^{b}$ (n=10) | $18.57\pm1.71^{n.s}$ (n=63) | $15.28\pm1.88^{n.s}$ (n=25) | $11.19\pm1.25^{n.s}$ (n=32) | $11.21\pm1.89^{n.s}$ (n=14) |
| 7 | $33.09\pm1.30^{a}$ (n=23) | $29.16\pm1.58^{b}$ (n=8) | $16.56\pm1.54^{a}$ (n=52) | $11.76\pm1.94^{b}$ (n=21) | $10.46\pm1.51^{n.s}$ (n=26) | $10.02\pm1.29^{n.s}$ (n=10) |

All values are given as mean ± s.e. Different superscript alphabets in the same column of shoots dimensions within same harvesting week indicate significant difference at $p<0.05$ (ANOVA, t-Test), i.e., a > b; n.s is not significantly different.

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**Table 5: The agronomic comparison of yield production for *L. flava.***

| Culture method | Plant material | Fresh weight ($g/m^2$) | Yield ($shoots/m^2$) | Number of bunch of harvested shoot ($bunch/m^2$) | Cropping season (month) | References |
|---|---|---|---|---|---|---|
| Inundated rice field, Indonesia | Seed | - | 20 | - | - | van den Bergh (1994) |
| Tank | Seed | 185.24 – 556.94 | 11 – 26 | 1 – 2 | 5 | This present study |
| Tank | Plantlet | 96.35 – 369.27 | 7 - 15 | 1 – 2 | 5 | This present study |