

RESEARCH ARTICLE

Agronomical and physiological characters of kaffir lime (*Citrus hystrix* DC) seedling under artificial shading and pruning

Rahmat Budiarto¹, Roedhy Poerwanto^{2,3*}, Edi Santosa^{2,3}, Darda Efendi^{2,3}, Andria Augusta⁴

¹Graduate School of Bogor Agricultural University, Bogor, 16680, Indonesia, ²Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia, ³Center for Tropical Horticulture Studies, Bogor Agricultural University, Indonesia, ⁴Research Center for Biology, Indonesian Institute of Science, Bogor, 16911, Indonesia

ABSTRACT

Kaffir lime (*Citrus hystrix* DC) is a minor citrus and its leaves are locally used for spices and essential oils. This study aimed to evaluate agronomical and physiological characters of kaffir lime seedling under modified cultural practices in order to improve leaf production. Shading and pruning were applied on five-month-old seedling during the rainy season, November 2017 to March 2018 in Bogor, Indonesia. The results showed that shading significantly improved the plant growth by 84%, and leaf number by 37%. Pruning treatment reduced the plant height by 8% and increased the shoot number by 20%. However, leaf production was more influenced by shading rather than pruning, with an increase for about 59% than control. This study also revealed a significant improvement on photosynthetic rate, stomatal conductance and water use efficiency on shaded lime for about 13%, 8% and 14%, respectively. Leaf pigment was also altered in response to shading, i.e the increasing chlorophyll B by 23% and the reduction anthocyanin by 38% on shaded lime. Mild shading with 24% light reduction is recommended to gain the leaf production of kaffir lime seedling.

Keywords: Chlorophyll; Pinching; Photosynthesis; Leaf production; Stomatal conductance

INTRODUCTION

Citrus has been recorded to be cultivated by the ancestors since 2100 BC so that it was called as ancient crop (Moore, 2001). Citrus is native to the southeast foothills of Himalaya prior to experience the species radiation in the end of Miocene era and then spread over tropical and subtropical regions worldwide (Wu et al., 2018). One of citrus species that is highly widespread in Southeast Asia, including Indonesia (Araujo et al., 2003) is kaffir lime (*Citrus hystrix* DC). De Candolle (DC) brought the seed of this lime from Mauritius and then classified it as the first species of papeda sub genus so that he called this lime as Mauritius papeda in 1824 (Swingle and Reece, 1967). This lime is also known by various names in its native area such as jeruk purut in Indonesia, kabuyaw in Philipinnes, limau purut in Malaysia, makrut in Thailand and truc in Vietnam (Wongpornchai, 2012).

Although the kaffir lime is not as popular as mandarin, oranges or pummelo, it still has a commercial value to be

developed worldwide, because its fragrant leaves can be commercialized as spices and essential oils (Toawatana et al., 2006; Khoe and Mi, 2015; Budiarto, 2018; Budiarto et al., 2019). Various potential of kaffir lime utilization should be followed with efforts to develop plant production. Unfortunately, there is no such reports due to the current status of kaffir lime as minor citrus that is apparently wild in much of central Malesia region (Mabberley, 2004) or even grow naturally by some villagers in their own backyard (Wongpornchai, 2012; Irsyam, 2015). To gain the leaf production, the kaffir lime should be intensively cultivated. The proposing agricultural inputs for leaf-oriented lime culture are shading and pruning, since two mentioned practices are frequently reported as plant growth regulation techniques.

Shading is one of culture techniques to reduce the sunlight exposure, air temperature and also evapotranspiration but increase the air relative humidity in the agroecosystem (Alarcon et al., 2006). In general, the reduction of

*Corresponding author:

Roedhy Poerwanto, Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia, Center for Tropical Horticulture Studies, Bogor Agricultural University, Indonesia. **E-mail:** roedhy8@yahoo.co.id

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sunlight exposure on shaded plants is estimated for about 15-39%, air temperature for about 2.3-2.5% and evapotranspiration for about 17-50%, followed with the improvement of air relative humidity for about 2-21%; however, it is highly influenced by the climatic region, shading structure, shading net type and also plant canopy (Mahmood et al., 2018).

Shading affect the plant growth and final yield either positive or negative, depending on the shading levels and plant sensitivity. Shading levels needs a lot of consideration in order to have beneficial stress, instead of harmful ones. Several negative effects of shading are delay the flowering of pineapple (Lin et al., 2015), lower the leafy production (Kosma et al., 2013), decline dry matter accumulation, flower and pod number of soybean (Bing and De-Ning, 2015; Bing et al., 2015), impede the apple fruit growth rate (Morandi et al., 2011), drop the fruit production and individual fruit size of lemon (Garcia-Sanchez et al., 2015). In contrary, beneficial effect of shading is also proved, i.e. the improvement of pitaya fruit color and also reduction of sunburn incidence (Chang et al., 2016), boosting the vegetative growth of several indigenous vegetables (Ekawati et al., 2010). Tolerant tomato varieties able to enlarge the fruit size by 7%, as the consequences of moderate shading, lower than 50% (Baharuddin, 2014).

Pruning is one of agriculture practices to control the plant growth, reduce the pest attack occurrence and also improve the effectiveness of horticultural management (Morales and Davies, 2000; Gilman and Black, 2011, Fake, 2012). For fruit-oriented production, pruning used to reduce the excessive vegetative growth, overcome alternate bearing habits and improve the fruit quality, while for ornamental plants pruning aimed to enhance the aesthetic quality of the plant (Joubert et al., 2000; Marini, 2014). Pruning is also capable to delay the flower induction (Budiarto, 2018) and also increases the number of leaves in young mandarin trees by 2-fold greater than unpruned ones (Septirosya, 2016).

Both shading and pruning treatment have been widely used individually in the number of fruit-oriented *Citrus* species, such as in acid lime (Ingle et al., 2001), grapefruit citrus (Sharma et al., 2007), lemon (Garcia-Sanchez et al., 2015; Gimeno et al., 2015), mandarin citrus (Singh et al., 2016), orange (Joubert et al., 2000), ponkan tangerine (Lee et al., 2015) and tangelo citrus (Morales and Davies, 2000). However, it has not been clearly reported on the leaf-oriented citrus species alike kaffir lime. Therefore, this article aimed to evaluate the agronomical and physiological characters of kaffir lime seedling under artificial shading and pruning for improving leaf production.

MATERIALS AND METHODS

The experiment was conducted in Pasir Kuda experimental farm of Bogor Agricultural University, Indonesia during the rainy season, November 2017 to March 2018. The latitude, longitude and altitude of the farm were -6.609042, 106.783605 and 263 meters above sea level, respectively. The soil of the farm was classified as latosol with clay texture. Soil pH was around 6.75 ± 0.46 . Soil moisture was depended on surface water especially rainfall. Irrigation was not applied during the experiment due the abundance of rainfall. During the experiment, monthly rainfall was ranged from 135.7 mm up to 339.3 mm (with an average of 219.6 mm).

Five-month-old kaffir lime seedlings grafted on Rangpur lime rootstock variety was used as plant material. All seedlings were disease free and relatively uniform in height and the number of leaves, i.e. 60 cm and 30 leaves, respectively. The seedling was arranged in nested design with two factors, namely shading and pruning. The first factor was shading that consisted of three levels, i.e. without shading (control), mild shading and moderate shading. Shading condition was created artificially by covering the shading frame (4 m x 1 m x 2 m) using black net (Surya, Indonesia) obtained from local market in Bogor, Indonesia. The net was set only to the roof for creating the mild shading treatment, while moderate shading meant the net fully covered the frame. The second factor was pruning that nested inside the first factor and consisted of two levels, i.e. control and pruning. Pruning, in form of pinching, was done by removing the upper shoots of the seedling. In average, the removed shoot consisted of six leaves or 20% of the total leaf number. In short, there were six combination treatments and replicated eight times for every treatment, so that was totally 48 seedlings evaluated in present experiment.

The experiment was started in November 1, 2017 with soil tillage operating manually by using a hoe. Seedlings were transplanted from the polybag into the land with spacing about 50 cm x 50 cm in November 15, 2017. The transplanted plants started to be exposed by artificial shading in December 1, 2017. Fertilizers at rate of 20 g N, 15 g P₂O₅ and 10 g K₂O were applied for individual lime through soil drench in December 15, 2017. Insecticide (Curacron, Indonesia) at rate of 1 ml per 1000 liter water were routinely sprayed once a month. Weeding was applied once a month manually by hand. Plant maintenance was carried out until the harvesting season, which was three months after treatment in the early March.

Microclimates variables such as the light intensity, the estimation of light reduction, the ambient temperature, soil

temperature and relative humidity were directly measured inside the shading frame once a week. The light intensity was measured at 01:00 p.m. in the sunny day by a Lux-28 portable digital lux meter (Danoplus, China) at the position of 10 cm above the apical bud of the plant (L_p) and above the soil surface below the plant canopy (L_b). The intercepted light (L_i) was determined by formula, i.e. $L_i = L_p - L_b$. The light reduction (L_r) was estimated by formula, i.e. $L_r = 100 \times (L_c - L_s) / L_c$. L_c was the light intensity in control treatment, while L_s was the light intensity in shading treatment. The ambient temperature and relative humidity were observed by thermo-hygrometer (Elitech, China). Soil temperature were measured by 4-in-1 soil survey instrument (Amtast, China). Additional microclimate variables such incoming radiation and leaf temperature were derived from Li-6400XT observation on 90 days after treatments (DAT).

Agronomical characters such as growth performances and plant production were mainly observed during the experiment. Growth performances such plant height, shoot number, leaf number, stem diameter, root volume, plant growth rate and dry biomass accumulation rate were measured at 90 DAT. Plant height could be measured with a ruler from the base of the stem above the soil up to the highest apical bud. The number of shoots and leaves were counted with a hand counter (Kenko, Jepang). Individual leaf area and leaf weight were measured with a Li-3000C portable leaf area meter (Licor Inc, USA) and analytical balance (Hwh, China), respectively. Stem diameter was measured with a digital calliper (Nankai, China). Root volume was obtained from the additional value of water inside the beaker glass after the dipped of root biomass. The rate of plant growth and dry biomass accumulation were calculated from the increasing amount of fresh weight and dry weight of the entire plants (comparing pre- to post-treatments) divided by the number of weeks needed, i.e. 12 observed weeks. The production of leaf, stem, root and entire plant were calculated from the fresh weight of each detached parts. Harvesting index (HI) was calculated by dividing the fresh weight of detached parts into the entire plant, so do the dry biomass partition (DBP) with the dry weight basis. To obtain dry weight and the water content, the samples were dehydrated in oven at 80°C for three days.

Physiological responses in form of photosynthetic rate, transpiration rate, stomatal conductance and leaf temperature were measured by using a Li-6400XT portable photosynthesis system (Licor Inc, USA) at 9:00 to 10:00 a.m. in the sunny morning of March 1 2018 or 90 DAT. Water use efficiency (WUE) was the ratio of photosynthetic rate to transpiration rate (Tuba et al., 1996). This measurement was involved four individual plants as replications for each combination treatment. For every plant, there were three leaves measured. The selected leaves

were green, disease-free and fully developed that located at the 5th and 6th position below the dormant apical bud.

Leaf pigments including anthocyanin, chlorophyll A and B were observed on 60 DAT, based on Sims and Gamon (2002). The leaf sample was fully developed and normal leaves that produced after the treatment, specifically at the 5th and 6th position below the dormant apical bud. The samples were directly packed into the cool box and transferred to laboratory. Fresh leaves weighing 0.02 grams were mashed with 2 ml acetris (85% acetone plus 1% tris) solution, and then centrifuged at 6000 rpm for 10 minutes. The supernatant as much as 1 ml was added with 3 ml of acetris for further mixed (Vortex, USA). Those solution was ready for absorbance measurement. The measurement was made at four wavelengths i.e. 470 nm, 537 nm, 647 nm and 663 nm by using a spectrophotometer. The pigment contents were further calculated by following equations.

1. Anthocyanin = $0.08173 \times A_{537} - 0.00697 \times A_{647} - 0.002228 \times A_{663}$
2. Chlorophyll A = $0.01373 \times A_{663} - 0.00089 \times A_{537} - 0.003046 \times A_{647}$
3. Chlorophyll B = $0.02405 \times A_{647} - 0.004305 \times A_{537} - 0.005507 \times A_{663}$
4. Total Chlorophyll = Chlorophyll A + Chlorophyll B

Leaf anatomy was also observed at 90 DAT using BX 51 light microscope (Olympus, Japan) calibrated on December 10, 2010, equipped with a 100x total magnification and integrated with DP2-BSW software computer to ease the anatomical measurement. The sample was prepared from fully developed, normal and damaged free kaffir lime leaf at the 7th position below the dormant apical bud. For every treatment, there were 3 leaves collected from 3 individual plants. All leaves were further processed by slicing a small part near the midrib of upper leaflet forming a small cube with a size of 0.5 x 0.5 cm. The sliced cube was transferred into object glass (Sail, China) and ready to be observed inside the light microscope. The object of measurement were the thickness of leaf and leaf composing tissue (um), including upper epidermal tissue, upper palisade, sponges, oil glands, lower palisade and lower epidermal tissue.

Analysis of variance was performed by using Statistical Analysis Software (SAS) version 9.4. For any significant differences between treatments was evaluated by Duncan Multiple Range Test (DMRT) at level of confident 5%.

RESULTS AND DISCUSSION

Microclimates variation

Microclimate conditions surrounding the treated kaffir lime were significantly affected by shading treatment

(Table 1). The direct effect of shading was the reduction of light intensity for about 24% in mild shading and 43% in moderate ones. The indirect effect was the reduction of ambient temperature for about 1.9-3.3°C, the reduction of leaf temperature for about 0.3-0.8°C, the reduction of soil temperature for about 2.3-4.3°C and the improvement of relative humidity for about 2-2.4%, compared to control. Microclimates modification through shading may have an effect on the plant production and quality (Mahmood et al., 2018). In agreement with previous work, present study also proved the difference growth, production and physiological response of kaffir lime in response to shading.

Growth performances

The growth of kaffir lime was improved by shading and pruning treatment (Table 2). Shading had a significant effect on plant growth rate, dry biomass accumulation rate, plant height, leaf number, stem diameter and root volume. Plant growth rates increased 89% in mild shading and 79% in moderate ones, compared to control. The rate of dry biomass accumulation and the height of plants increased 210% and 42% in both shading treatments, respectively. The leaf number of mild and moderate shaded lime were 38% and 35% greater than control, respectively. As compared to the control, mild shaded lime had 20% bigger stem and 74% bigger root, while moderate shaded ones had 13% bigger stem and 69% bigger root. Mild shading tended to be a slightly better than moderate ones in term of plant growth booster, however, both treatments were not significantly different in all variables, except the stem diameter. Shading solely was proved to be vegetative booster in numerous Citrus species, such as lemon (Gimeno et al., 2015), oranges (Incesu et al., 2016) and grapefruit (Cohen

et al., 2005). Beneficial effect of shading in Citrus might be related to the status of its species as C3 plant (Sage and Zhu, 2011), since shading was inauspicious for C4 plants (Mahmood et al., 2018).

In other hand, pruning treatment significantly affected the height and the number of shoots of lime. The height of pruned plants was 8% lower than the unpruned ones since the detached amount of shoot at the initial time. The reduction of plant height could be useful to ease the plant maintenance and leaf harvesting activity. Pruning could increase almost 20% of the number of shoots of kaffir lime. The greater shoot number on pruned kaffir lime caused by the reduction of apical dominance as the shoot apical was pinched. Manual or chemical treatment to reduce the apical dominant was successfully proved to stimulate rapid new flush growth in several citrus (Sharma et al., 2007; Aliyah et al., 2015; Singh et al., 2016), since the plant wanted to immediately restore the lost foliage (Phillips, 1978). The greater shoot number, the higher potential for plant to have more leaf. Although it was not significantly different, the pruned lime tended to have more leaves than unpruned ones.

Pruning was not significantly affected the area of and weight of individual leaf of kaffir lime (Table 3). Plant response to pruning was varied depend upon the pruning level applied. Heavy pruning in form of removal the entire existing canopy of 4-year-old mandarin citrus caused the decline of source capacity so the plant was only able to produce the smaller leaf size than the unpruned ones (Budiarto, 2018). Yuan et al. (2005) also reported that defoliation, another term of pruning with relatively similar

Table 1: Microclimate variation under different shading treatments during study period

Microclimates	Control	Mild shading	Moderate shading
1. Light intensity (x 1000 lux)	152.21±2.68a	115.80±3.84b	86.59±3.75c
2. Incoming radiation (W m ⁻²)	160.11±0.10a	159.96±0.16b	159.81±0.05c
2. Ambient temperature (°C)	29.50±0.93a	27.63±1.06b	26.25±0.71c
3. Leaf temperature (°C)	29.03±0.30a	28.70±0.51b	28.21±0.39c
4. Soil temperature (°C)	34.50±0.71a	32.25±0.46b	30.25±0.71c
5. Ambient relative humidity (%)	71.63±1.77b	73.63±1.06a	74.00±1.20a

Means in the same row followed by different alphabets are significantly different based on DMRT at α 5%.

Table 2: Growth performance of kaffir lime in response to shading and pruning at 90 DAT

Treatments	Plant growth rate (g week ⁻¹)	Dry biomass accumulation (g week ⁻¹)	No. shoot	Plant height (cm)	No. leaf	Stem diameter (cm)	Root volume (ml)
Shading							
No	5.53±0.40b	1.20±0.35b	4.25±0.50	70.9±2.3b	75.8±2.5b	0.98±0.02c	14.86±1.62b
Mild	10.21±0.35a	3.80±0.45a	4.38±0.54	101.1±2.7a	104.6±5.9a	1.18±0.02a	25.86±1.25a
Moderate	9.88±0.59a	3.65±0.40a	4.50±0.82	100.4±3.1a	102.0±2.7a	1.11±0.02b	25.13±1.40a
Pruning							
No	8.31±0.50	3.09±0.40	4.00±0.61b	94.8±2.5a	92.2±3.5	1.09±0.02	21.17±1.34
Pruning	8.77±0.40	3.20±0.40	4.75±0.63a	86.8±2.4b	96.1±3.4	1.09±0.02	22.75±1.50
CV (%)	3.54	6.61	13.99	2.11	3.7	1.45	4.59

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

meaning, should not exceed 25% of the total canopy in order to prevent the negative impact on leaf size, canopy growth, fruit yield and quality. It was likely that citrus was sensitive to leaf disturbance, so the pruning level should be adjusted to be lighter in order to have beneficial stress instead of harmful ones. Therefore, present study applied the light level of pruning since only 1/5 of the seedling foliage removed.

In opposite to pruning, shading treatment was success to significantly enlarge the individual leaf size of kaffir lime (Table 3). The shaded lime had 27% larger leaf than exposed lime. The enlargement of leaf surface is frequently happened under shaded condition as plant wanted to expand the sunlight harvesting area (Huxley, 1967, Levitt, 1980; Matsoukis et al., 2015). Plant grown on natural open environment maintained the leaf size in a very strict way

Table 3: Individual leaf size of kaffir lime in response to shading and pruning at 90 DAT

Treatments	Individual leaf size	
	Leaf area (cm ²)	Leaf weight (gram)
Shading		
No	18.38±0.35b	0.55±0.01b
Mild	23.32±0.70a	0.70±0.02a
Moderate	23.49±0.90a	0.71±0.03a
Pruning		
No	22.14±0.60	0.66±0.02
Pruning	21.33±0.73	0.64±0.02
CV (%)	1.72	2.01

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

to keep the balance of sink and source. The greater leaf area on the exposed lime was beneficial for the growth; however, it showed the greater transpiration (Zlatev and Lidon, 2012). Under shading treatment, the transpiration is minimized so the water loss due to the greater leaf area could be managed properly. Shading produced not only a larger leaf but also a heavier leaf with a significant improvement by 28% compared to exposed lime. This finding might be associated with the absence of significant reduction on leaf thickness.

Pruning treatment was not show any significant effect on the thickness of individual leaf and its constituent tissues, so did the shading (Table 4). However, there was a tendency for the reduction in leaf thickness and its constituent tissues such as epidermal and palisade tissue, due to the artificial shading. Leaf thickness reduction was one of well-known adaptation strategy of plant under shading condition (Vogelman and Martin, 1993; Anderson et al., 1995; Taiz and Zeiger, 2002). A less thick of shaded leaves caused by underdevelopment of mesophyll cell was beneficial to minimize the cost of cell maintenance (Grecco et al., 2014).

Plant production

Production and estimated productivity of kaffir lime were also significantly affected by shading and pruning treatments (Table 5). The fresh weight of entire plant was significantly increased by shading for about 58% and pruning for about 4%. The stem production also increased by 58% and 8% due to the shading and pruning, respectively. The leaf and

Table 4: The thickness (μ m) of individual kaffir lime leaf and its constituent tissues following shading and pruning at 90 DAT

Treatments	Upper epidermis	Lower epidermis	Upper palisade	Lower palisade	Sponge	Oil gland	Total
Shading							
No	18.56±3.9	22.48±4.8	36.73±8.0	83.30±8.1	179.19±17.8	149.91±28.4	356.82±17.3
Mild	16.69±2.4	19.02±6.8	32.40±7.8	75.04±11.7	168.74±21.3	140.48±19.7	326.67±20.7
Moderate	15.52±4.6	20.61±7.6	34.24±2.1	61.61±14.6	182.81±21.3	156.14±38.5	328.54±24.9
Pruning							
No	16.96±4.1	22.5±4.7	35.03±5.7	72.37±12.8	178.16±22.4	144.63±19.1	336.54±25.3
Pruning	16.89±2.9	18.9±8.1	33.89±6.3	74.26±10.2	175.67±17.9	153.05±38.6	338.15±16.7
CV (%)	22.63	31.73	22.08	17.16	5.35	19.09	6.18

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

Table 5: Production of kaffir lime following shading and pruning at 90 DAT

Treatments	Production (g)				Estimated Productivity(ton ha ⁻¹)	
	Plant	Leaf	Stem	Root	Leaf	Stem
Shading						
No	92.72±4.91b	40.98±1.16c	35.86±1.82b	15.88±2.15b	1.64±0.04c	1.43±0.12b
Mild	148.91±4.97a	66.78±1.59a	57.10±1.57a	25.04±2.13a	2.67±0.06a	2.28±0.10a
Moderate	145.01±5.05a	63.73±1.44b	55.87±1.51a	25.41±2.36a	2.55±0.05b	2.23±0.08a
Pruning						
No	126.10±5.14b	57.07±1.46	47.67±1.74b	21.36±2.17	2.28±0.49	1.91±0.42b
Pruning	131.66±4.81a	57.26±1.33	51.55±1.53a	22.86±2.26	2.29±0.47	2.06±0.39a
CV (%)	2.81	1.73	2.52	7.87	1.73	2.52

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

root fresh weight was more influenced by shading rather than pruning, with an increase of both variables by 59% compared to the exposed lime. Unlike the root, leaf and stem are two important commercialized parts of kaffir lime agribusiness in Indonesia. The leaf is mostly sold as cooking spices at a price of IDR 20,000 kg⁻¹, while the stem is a post-harvest by-product that is bought by essential oil refineries as fuelwood at a price of IDR 1,000 kg⁻¹ (Budiarto et al., 2019). The productivities of leaf and stem could be estimated by multiplying the individual yield with the number of plant population per ha (40,000 plants with a planting distance for about 50x50 cm). The greater the individual yield, the higher estimated productivity. Under shaded condition, the leaf productivity is increased by 0.97 ton ha⁻¹. The improving production and hence estimated productivity on shaded lime was caused by the improving growth performances as previously reported. Similar finding on indigenous vegetables showed by Ekawati et al. (2010) that artificial shading able to double the yield of *Talinum triangulare* and to quadruple the yield of *Pilea trinervia*.

Both shading and pruning were not caused a significant difference on harvesting index, dry biomass partition and water content of each plant parts (data not shown). However, those variables were seemed to be altered when compared between pre- and post-treatment (Fig. 1). As

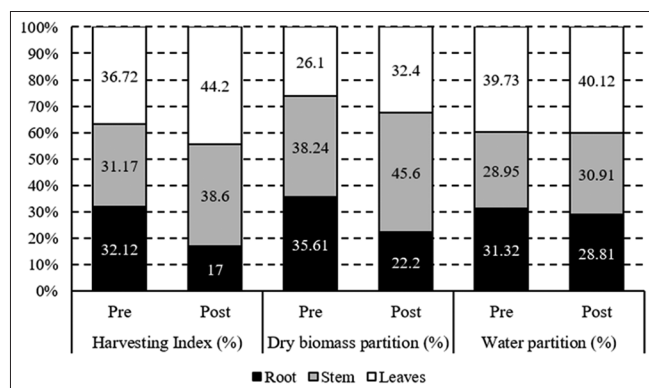


Fig 1. Percentage of harvesting index, dry biomass and water partition of kaffir lime at pre- and post-treatments.

the running of time, the seedling would grow to be young plant that experienced an improvement of canopy growth more dominant than the root ones. Rapid canopy growth aimed to enlarge the source capacity of the plant. A less dominance of root was associated with the adequate of water surrounding the plant during the study period, i.e rainy season. The plant tended to boost the canopy growth rather than its roots under favourable conditions (Zlatev and Lidon, 2012). The stem became the most dominant part in term of dry biomass, with an increase of 7.4%, compared to pre-treatment. Under favourable condition, it was common fact that the plant allocated a greater amount of biomass into the stem lead to the abundance lignocellulosic material in the stem for strengthening the plant structure.

Plant photosynthesis

Most of the physiological responses of kaffir lime were significantly affected by shading solely (Table 6). Although the shading screen had reduced the light intensity, it still sufficient for citrus leaves to reach maximum assimilation rates (Jifon and Syvertsen, 2003). There was a significant improvement in photosynthetic rate on shaded trees for about 13% than control. The higher photosynthetic rate on shaded lime is associated with better growth performances and subsequently plant production. This finding on kaffir lime was in agreement with previous studies on numerous Citrus species such as orange (Medina et al., 2002), grapefruit (Cohen et al., 2005), tangor citrus (Raveh et al., 2003) and lemon (Alarcon et al., 2006).

Moreover, shading could help the citrus leaf to avoid the incidence of photoinhibition. Photoinhibition is the reduction of solar energy conversion efficiency caused by the excessive photon absorption. The light saturation point for citrus is about 400 mmol m⁻² s⁻¹ (Syvertsen (1984). Exceeding the saturation point could promote the accumulation of unused excitation energy and then lead to photoinhibition. Previous studies reported that shaded leaves were less photoinhibited than sunlit leaves, leading to the higher photosynthetic efficiency (Jifon and Syvertsen, 2003).

Table 6: Physiological responses of kaffir lime following shading and pruning

Treatments	Photosynthetic rate (μmol CO ₂ m ⁻² s ⁻¹)	Stomatal conductance (mol H ₂ O m ⁻² s ⁻¹)	Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)	Water use efficiency (μmol CO ₂ mmol H ₂ O ⁻¹)
Shading				
No	28.93±2.45b	0.66±0.02c	10.56±0.02a	2.74±0.23b
Mild	30.57±3.08b	0.69±0.05b	10.48±0.02b	2.92±0.30b
Moderate	34.53±3.65a	0.73±0.04a	10.42±0.01c	3.31±0.35a
Pruning				
No	30.68±2.89	0.70±0.04	10.47±0.01b	2.93±0.28
Pruning	32.01±3.23	0.68±0.03	10.50±0.02a	3.05±0.31
CV (%)	9.98	6.44	0.08	10.05

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

Table 7: Leaf pigmen of kaffir lime following shading and pruning

Treatments	Chlorophyll			Anthocyanin (mg 100g ⁻¹ sample)	
	A (mg g ⁻¹)	B (mg g ⁻¹)	A/B	Total (mg g ⁻¹)	
Shading					
No	1.33±0.031	0.42±0.017c	3.16±0.127a	1.75±0.034b	0.077±0.005a
Mild	1.31±0.039	0.49±0.014b	2.69±0.121b	1.79±0.040ab	0.057±0.002b
Moderate	1.30±0.051	0.54±0.031a	2.41±0.184c	1.84±0.041a	0.055±0.008b
Pruning					
No	1.32±0.039	0.48±0.021	2.78±0.366	1.80±0.034	0.063±0.005
Pruning	1.30±0.042	0.48±0.020	2.73±0.349	1.79±0.043	0.062±0.006
CV (%)	2.73	4.67	5.03	2.34	7.50

Means in the same column followed by different alphabets are significantly different based on DMRT at α 5%; CV-coefficient of variation.

The higher photosynthetic rate on shaded lime is resulted by the higher stomatal conductance. Stomatal conductance is a term for the rate of CO₂ entering and water vapor exiting through the stomatal pore of the leaf. As the stomatal conductance increased, the CO₂ concentrations at the chloroplasts also increased (Raveh et al., 2003). Present study confirmed that the shaded leaf stomatal conductance was 8% greater than exposed ones. Haijun et al. (2015) reported that the lower evaporative demand under shading condition facilitated the plant to improve the stomatal conductance and then photosynthetic rate.

Citrus plant was naturally exposed with transient water deficit at the midday and subsequently caused the lower stomatal conductance and also net assimilation rate (Alarcon et al., 2006). The reduction of direct radiation through shading treatment could help the plant to prevent the transient water deficit (Medina et al., 2002; Cohen et al., 2005), so that it can improve the integrated daily net CO₂ uptake for about 20% greater than the sunlit tree (Alarcon et al., 2006). The water deficit could be stimulated by the higher temperature during the midday that boost plant transpiration rate.

Both pruning and shading have significant effect on transpiration rate of kaffir lime (Table 6). Transpiration rate was significantly improved by pruning, while shading seemed to reduce it. More leaves produced by pruned lime was attributed to the higher transpiration. In opposite, lower transpiration on shaded tree was commonly reported because of the limited solar radiation lead to the alteration of microclimate surrounding the plant. Under moderate shading condition with 43% light reduction, the lower the temperature by 0.82°C, the lower transpiration rate by 0.14 mmol H₂O m⁻² s⁻¹, compared to the control. The reduction of transpiration on shaded plant was followed with the improvement of water use efficiency (Nicolas et al., 2008). Our finding revealed that shaded lime had 14% greater water use efficiency than the control. It was in similar to previous study on young lemon by Alarcon et al., (2006).

E. Leaf pigment

Shading, rather than pruning, had a significant effect on the pigment content of kaffir lime leaves, except the chlorophyll A content (Table 7). Shading treatments significantly increased the chlorophyll B and total chlorophyll content by 23% and 4%, but it was also decreased the ratio of chlorophyll A to B and anthocyanin content by 19% and 38%, respectively. The higher the shading level tested in present study, the greater the value of chlorophyll B and total chlorophyll, the lower the content of accessory pigments such anthocyanins. Previous study on Ponkan citrus also showed similar finding (Grecco et al., 2014).

A higher chlorophyll B content, followed with lowering the ratio of chlorophyll A to B, was indicator of plant strategy adapted to the shading, since the enhancement of the size of the second photosystem antennas lead to have a more efficient photosynthesis and also reduce the photo-damage resulted (Hooper and Eggnik, 2001; Pattanayaka et al., 2005; Yamasato et al., 2005). The reduction of anthocyanins content was aimed to enhance the light harvesting efficiency (Levitt, 1980) because sometime this pigment able to impede the running of photosynthesis by its accumulation in vacuoles of epidermis tissue (Sopandie, 2013). Our finding was also in agreement with Kosma et al. (2013) who stated that the higher pigment content on shaded plant was associated to better grana development, more chloroplasts, more chlorophylls per chloroplast as efficient plant adaptation strategy under the shading.

CONCLUSION

Kaffir lime seedling experienced better growth performances under shading condition that indicated by the improvement of plant growth by 84%, dry biomass accumulation by 210%, leaf number by 37%, stem diameter by 17%, root volume by 72%, leading to better leaf production by 59% than unshaded ones. Pruning was significantly increased the shoot number by 20%, however it showed no significant improvement of leaf production, as shading did. Individual leaf size was increased by shading, while pruning seemed

to have no effect. Shaded lime growth improvement was attributed to the increase of photosynthetic rate, stomatal conductance, water use efficiency by 13%, 8% and 14% greater than the control, respectively. Shaded leaves showed greater chlorophyll B by 23%, lower anthocyanin by 38% than unshaded ones, indicated the physiological adaptation strategy to have a more efficient photosynthesis under shaded condition. Mild shading with 24% light reduction is highly recommended rather than moderate shading with 43% light reduction because it sounds more effective and efficient to boost the leaf production of kaffir lime seedling.

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Authors' contribution

R.B. and R.P. planned the experiment. R.B. carried out the experiments. E.S. improved the number of variables observed. D.E. contributed to field management. R.B. and R.P. wrote the manuscript by considering critical feedbacks from E.S., D.E. and A.A.

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