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Article

The Increase in Kaffir Lime Leaf Production Due to Gibberellin Is Diminished by Pruning

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Abstract: Gibberellin regulates the flowering and fruiting response of fruit-oriented citrus, but there are a lack of studies on the methods for boosting the production of leaf-oriented citrus. This study aimed to analyze the effects of exogenous gibberellins and pruning on the leaf production of kaffir lime. Kaffir lime seedlings, grafted into rangpur lime, were arranged in a split-plot design under an open field condition. Four gibberellin concentrations (control, 0.005%, 0.01%, and 0.02%) and two pruning levels (no pruning and pruning at 30 cm main stem) were subjected to selected seedlings. The result showed a contrasting effect between gibberellin and pruning on the plant height and shoot number. Pruning significantly reduced the flush number, flush length, leaf length, leaf area, and leaf weight by 38%, 44%, 17%, 26%, and 28%, respectively. Without pruning, applying 0.02% gibberellin could produce the longest flush, with an increase in the number and weight of leaves by about 77% and 64%, compared with the control, respectively. Multivariate analysis also confirmed the positive effect of gibberellin on leaf production. However, these positive results are diminished by pruning due to limited source capacity, as indicated by the fewer leaves. Future research is required to analyze whether increasing gibberellin concentrations can speed up the recovery of post-pruned kaffir lime.

Keywords: *Citrus hystrix*; citrus; flushing; gibberellic acid; leaf disturbance; source capacity

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1. Introduction

Kaffir lime (*Citrus hystrix* DC) is a minor citrus that is apparently wild and frequently used as a leaf spice in numerous Asian dishes [1–3]. Southeast Asia is predicted as being the origin of this lime [4]. Compared to fruit-oriented species, like mandarin (*C. reticulata*), pomelo (*C. maxima*), lemon (*C. limon*), and orange (*C. sinensis*), the kaffir lime is less popular to cultivate [5]. However, this species displays a unique bifoliate leaf that is easily recognized [6,7] and contains numerous volatile metabolites, which are incorporated into essential oils [8,9]. The essential oil of kaffir lime can be extracted from leaves, peel, flowers, and twigs; it is multipurpose and can be used in both the food and pharmaceutical industries [10,11].

Aside from essential oils, the main economic organ of kaffir lime cultivation is its aromatic leaves [3], with the major metabolites being citronellal [10]. Citronellal-rich leaves display various beneficial properties, i.e., an intense lemony scent [12,13], antifungal [14,15], antibacterial [16], anti-inflammatory [17], and mosquito-repellent [18–20], and they are the raw material for synthesizing citronellol, isopulegol, and menthol [21–23]. Numerous beneficial properties of kaffir lime leaves make them a promising commodity for the global growing market. However, the supply of leaves is predominantly fulfilled by local farmers who culture them less intensively in the yard [1,3].

Intensification is the alternative solution to increase plant production amidst the difficulty of land expansion [24]. Agricultural intensification can be performed in numerous ways, from basic approaches, such as plant growth regulator (PGR) application [25] and manure nutrient management [26], to sophisticated ones, such as genomic-assisted breeding [27]. One of the popular PGRs in citrus fruit farming is gibberellin, as applied in the form of gibberellic acid [28].

Gibberellin is isolated from *Gibberella fujikuroi* (recently known as *Fusarium fujikuroi*) [29]. Gibberellin application regulates the flowering gene, leading to the floral determinacy of oranges (*C. sinensis*) [30]. Gibberellin also overcomes the problem related to alternate bearing on citrus fruits by forming a more uniform fruit yield year by year, increasing the fruit in the off-year, and limiting the fruit in the on-year through flowering intensity and crop load regulation [31–34]. In terms of post-harvest, gibberellin can be used to delay citrus fruit color break [35], enhance the effectiveness of post-harvest disease control [36], and increase citrus fruit juice content [37,38]. However, there are still a limited number of studies regarding the effect of gibberellin on leaf-oriented citrus genotypes like kaffir lime.

Applying exogenous gibberellins to kaffir lime is potentially required, since previous studies by [39,40] have reported the success of gibberellin in supporting leaf expansion [41,42] and alleviating stress severity. Leaf-oriented citrus genotypes, like kaffir lime, are frequently exposed to stress due to being subjected to pruning as part of the harvesting activity [1,43]. Pruning can actually bring about benefits, such as eliminating pest-attacked foliage and regulating flowering and alternate bearing [44], but uncontrolled pruning, i.e., too severe, may become a leaf disturbance stress that interferes with the balance of the plant source and sink, leading to a decline in plant growth and yield [44,45]. This study intends to analyze the effects of exogenous gibberellins on leaf production in pruned and unpruned kaffir limes.

2. Materials and Methods

2.1. Planting Materials

Kaffir lime scion grafted onto rangpur lime (*C. limonia* Osbeck) rootstock was prepared for the experiment. Five months after grafting, the seedling was transplanted to the open field at Bale Tatanen Padjadjaran, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, Indonesia (−6.9316851, 107.775863, 718 m above sea level), in January 2023. Four months later, the initial selection was performed to eliminate the abnormal seedling, resulting in normal seedlings being subjected to treatment from May to July 2023. The climate characteristics during the growing periods (January to May) included a daily temperature of 26–28 °C and a relative humidity of 46–53%. The edaphic characteristics of the growing sites were inceptisol soil order, with a dominant clay texture that was enriched with organic material in the form of goat manure at the beginning of planting. The characteristics of the selected seedlings were normal growth, bifoliate leaves, pest- and disease-free, and relatively uniform in terms of single shoot, leaf number (±75 leaves), and plant height (±75 cm).

2.2. Research Procedure

This experiment was arranged in a split-plot design with two factors, namely gibberellin concentration and pruning. The four gibberellin concentrations were control, 0.005%, 0.01%, and 0.02%, which were prepared from diluted commercial gibberellin tablets of 0.00 g, 0.01 g, 0.02 g, and 0.04 g (with 20% gibberellic acid as the active ingredient) in 100 mL water, respectively. The two pruning levels were control (no pruning) and pruning at 30 cm above the joining grafting spot, as recommended by an earlier study [45]. All of the combination treatments were replicated five times. The agricultural maintenance included fertilizer and gibberellin application, weeding, pest control, and harvesting. Inorganic fertilizer in the form of NPK 25 g/plant was applied periodically via soil drench.

According to the adjusted treatment, gibberellic acid was applied weekly for up to seven weeks after treatment (WATs). Hand weeding was carried out monthly to control weed growth. Pest control was initiated by pest inspection once a week and then chemically controlled. Harvesting was conducted at 8 WATs by a pruner to cut only the newly emerged flush in the post-treatment period.

2.3. Data Mining and Analysis

The growth and production variables of kaffir lime were measured. Plant height and shoot number were measured periodically every two weeks, from 2 to 6 WATs, using a hand counter and roll meter. The flush number and flush length were measured on the harvest day using a hand counter and roll meter. The proportion of branches on the flush was calculated by dividing the branch weight by the entire flush weight, and so was the proportion of leaves. The leaf length was measured and became the input for predicting the leaf area and individual leaf weight, following a previous study [6]. Leaf weight and leaf number were also measured per plant using an analytical balance and hand counter. All of the obtained data were subjected to analysis of variance followed by Tukey test at $\alpha = 0.05$ using Minitab. Additionally, multivariate analysis in the form of correlation and regression was performed using RStudio 2023.06.2+561 and Microsoft Excel Professional Plus 2016, respectively.

3. Results

3.1. Contra Effect between Gibberellin and Pruning on the Plant Height and Shoot Number

Both gibberellin and pruning significantly impact kaffir lime growth, as indicated by the increase in plant height ($p < 0.05$) and shoot number ($p < 0.05$) from 2 to 6 WATs (Table 1). Applying 0.005% gibberellin resulted in the significantly tallest plant, with an increase of 21%, 13%, and 12%, compared with no gibberellin at 2 WATs, 4 WATs, and 6 WATs. Plants treated with gibberellin concentrations of 0.01% and 0.02% produced a similar pattern of plant height improvement to what the control did. Applying gibberellin also significantly influenced the shoot number, but the pattern was more varied. At 2 WATs, the effect of gibberellin on the number of shoots was insignificant. At 4 WATs, the highest number of shoots was observed in plants with 0.02% gibberellin concentration. However, two weeks later, at 6 WATs, the number of shoots in that treatment became the lowest and significantly different compared to the control, with a difference of 61.5%.

Table 1. Kaffir lime growth, i.e., plant height and shoot number, in response to various gibberellin and pruning applications.

Treatment	Plant Height (cm)			Plant Shoot Number (Shoots)		
	2 WATs	4 WATs	6 WATs	2 WATs	4 WATs	6 WATs
Gibberellin (G) factor						
0.000%	50.67 b	65 b	66.67 b	3.5 a	5.17 ab	8.67 a
0.005%	61.5 a	73.5 a	76 a	4.33 a	5 ab	4.17 b
0.01%	55.83 b	64.33 b	64.17 b	4 a	4 b	7.5 a
0.02%	53.08 b	64.67 b	66.17 b	6.17 a	6.17 a	3.33 b
Pruning (P) factor						
No pruning	75.42 a	84 a	85.17 a	4.58 a	6.17 a	4 b
Pruning	35.13 b	49 b	51.33 b	4.42 a	4 b	7.83 a

Note: WATs—weeks after treatment. Mean values within the same column and same factor followed by a similar letter are not significantly different based on the Tukey test at $\alpha = 0.05$.

Concerning pruning, the unpruned limes showed a significantly taller stem ($p < 0.05$) than the pruned ones at all observation times, with a reduction in plant height of about 53%, 42%, and 40% at 2 WATs, 4 WATs, and 6 WATs. A significant reduction in plant shoot number was also noted in the effect of pruning at 4 WATs; however, the opposite result was noticed at 6 WATs, where the pruned limes had more shoots compared to the unpruned ones and this was significant (Table 1).

3.2. The Effect of Pruning Is More Dominant Than Gibberellin on Flushing Performance

Flushing is an emerging process where a series of young small leaves develop as a bud grows and develops in a certain time and under a certain condition. A single newly emerged flush is composed of a certain portion of branches and leaves. Statistical analysis resulted in the argument that pruning significantly affected the proportion of newly emerged flush ($p < 0.05$). The present findings confirm that leaves contributed in part to 62–69%, while the rest was due to the branch portion (Figure 1). The leaf portion in the pruned plants was significantly lower than in the unpruned ones; thus, the branch portion in the pruned limes was greater than in the unpruned limes. In contrast, the proportion of leaves and branches was not significantly affected ($p > 0.05$) by gibberellin application.

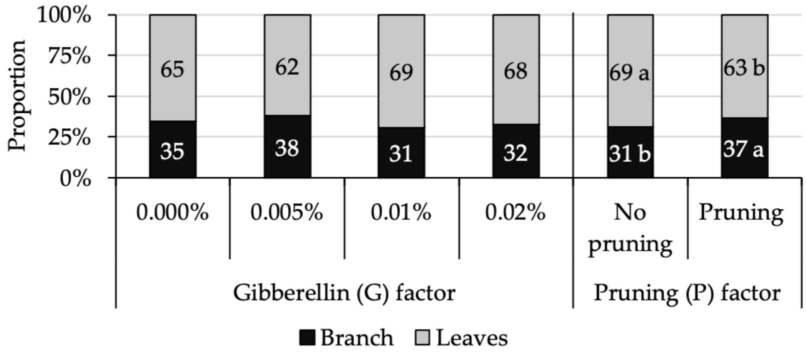

Figure 1. Proportion of branches and leaves in the newly emerged flushes of kaffir limes under different levels of gibberellin and pruning application. Note: mean values within the bar of the same factor followed by a different letter are significantly different based on the Tukey test at α = 0.05.

Applying gibberellin also did not significantly affect the flush number, leaf area, and individual leaf weight (Table 2). However, applying 0.02% gibberellin produced the longest flush that was significantly different ($p > 0.05$) from the other gibberellin concentrations, as clearly depicted in G3 of the unpruned plant (Figure 2A); however, this was not significantly observed for the pruned plant (Figure 2B). Aside from gibberellin, pruning showed a more dominant effect on the flushing response of the kaffir lime plants. The presence of pruning resulted in a significantly lower flush number, flush length, leaf length, and leaf area ($p < 0.05$) than the unpruned ones. The reduction in flush number, flush length, leaf length, and leaf area was 38%, 44%, 17%, 26%, and 28% due to pruning, respectively.

Table 2. The number, length, and weight of newly emerged flush on kaffir limes in response to different levels of gibberellin and pruning application.

Treatment	Flush Number (Flush)	Flush Length (cm)	Leaf Length (cm)	Leaf Area (cm^2)	Individual Leaf Weight (g)
Gibberellin (G) factor					
0.000%	6.33 a	18.58 b	10.05 b	26.34 a	0.79 a
0.005%	4.50 a	22.67 b	10.55 ab	29.69 a	0.94 a
0.01%	4.83 a	19.75 b	11.38 a	31.41 a	1.05 a
0.02%	6.00 a	31.50 a	10.70 ab	28.43 a	0.86 a
Pruning (P) factor					
No pruning	6.67 a	29.58 a	11.66 a	33.27 a	1.06 a
Pruning	4.17 b	16.67 b	9.68 b	24.66 b	0.76 b

Note: mean values within the same column (B) of the same factor followed by a similar letter are not significantly different based on the Tukey test at α = 0.05.

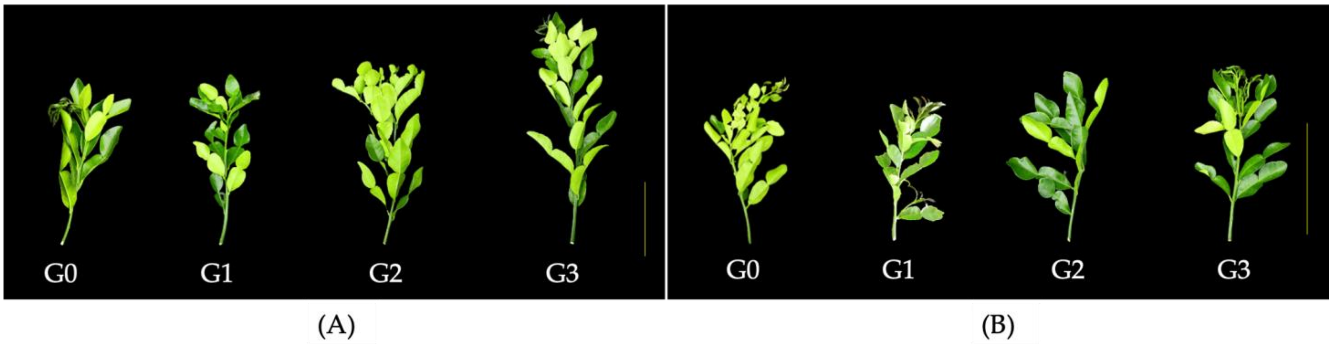

Figure 2. Newly emerged flush in unpruned (**A**) and pruned (**B**) kaffir limes in response to various concentrations of gibberellin applied. Note: yellow line in both figures is equal to 10 cm. G0—0.000%, G1—0.005%, G2—0.01%, and G3—0.02%.

3.3. The Increase in Leaf Production Due to Gibberellin Is Diminished by Pruning

Due to its importance as a leaf spice commodity, the production of kaffir lime is targeted to leaf organs rather than fruit ones. Leaf production, as indicated by leaf number and leaf weight, was significantly affected by gibberellin only in unpruned limes ($p < 0.05$) (Figure 3A,B), while the effect on the pruned ones was insignificant ($p > 0.05$) (Figure 3C,D). Applying gibberellin at 0.005% and 0.01% resulted in similar results to those with no gibberellin applied; however, the highest concentration applied, i.e., 0.02%, produced a significantly higher leaf number and leaf weight than the control. The increase in the number and weight of leaves was seen, as the effect of 0.02% gibberellin on unpruned plants was 77% and 64%, compared with the control, respectively.

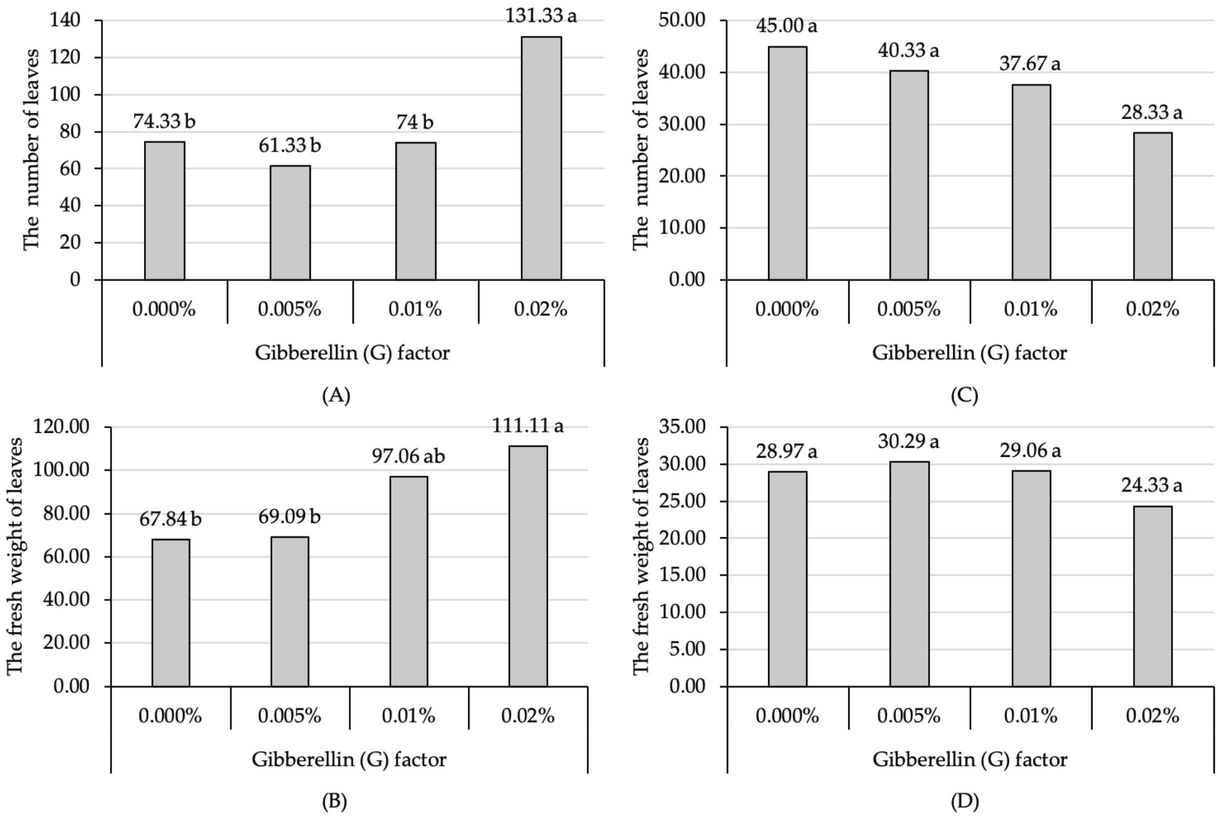

Figure 3. Yield component of kaffir limes in response to various concentrations of gibberellin applied: the number of leaves in unpruned (**A**) and pruned kaffir limes (**C**) and the weight of leaves in unpruned (**B**) and pruned kaffir limes (**D**). Note: mean values above the bar in the same chart followed by a similar letter are not significantly different based on the Tukey test at $\alpha = 0.05$.

3.4. Multivariate Analysis Confirmed the Positive Effect of Gibberellin on Leaf Production of Unpruned Plants

The positive effect of gibberellin in increasing kaffir lime leaf production was validated via multivariate analysis. Pearson analysis revealed 30 significant correlations and 123 insignificant correlations among 17 variables observed (Figure 4). Gibberellin displayed the following: (i) a positive and significant correlation with flush length (r 0.86), leaf number (r 0.78), and leaf weight (0.83) and (ii) a negative and significant correlation with shoot number (r −0.78). Additionally, the leaf length was positively significantly correlated (r 0.89) with the leaf area, irrespective of gibberellin concentration, while the leaf portion was negatively correlated with the branch portion in the newly emerged flush.

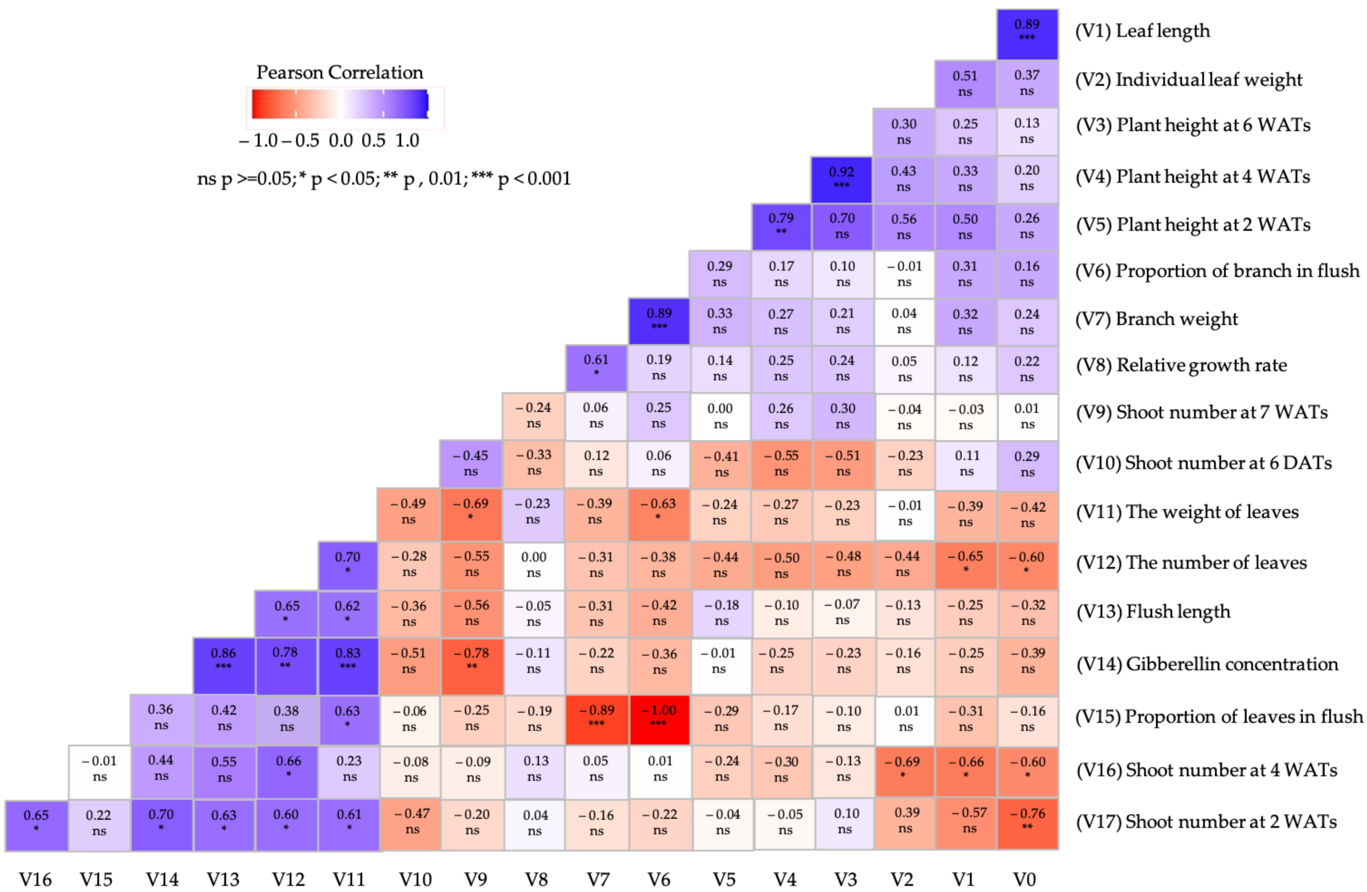

Figure 4. Pearson correlation analysis between gibberellin concentration, characteristics of growth, and production of unpruned kaffir limes.

Aside from correlation, the regression test elucidated the mathematical equations and coefficient determination (R^2) of three models connecting (i) gibberellin and leaf number; (ii) gibberellin and leaf weight; and (iii) gibberellin and flush length (Figure 5). All quadratic regression models displayed strong coefficient determination (higher than 90%), implying that the high power of a certain variable can be predicted using the mathematical equation, leading to narrow differences between the fitted and observed data. In the absence of pruning, increasing the gibberellin concentration (up to 0.02%) applied can lead to an improvement in flush length, leaf number, and weight.

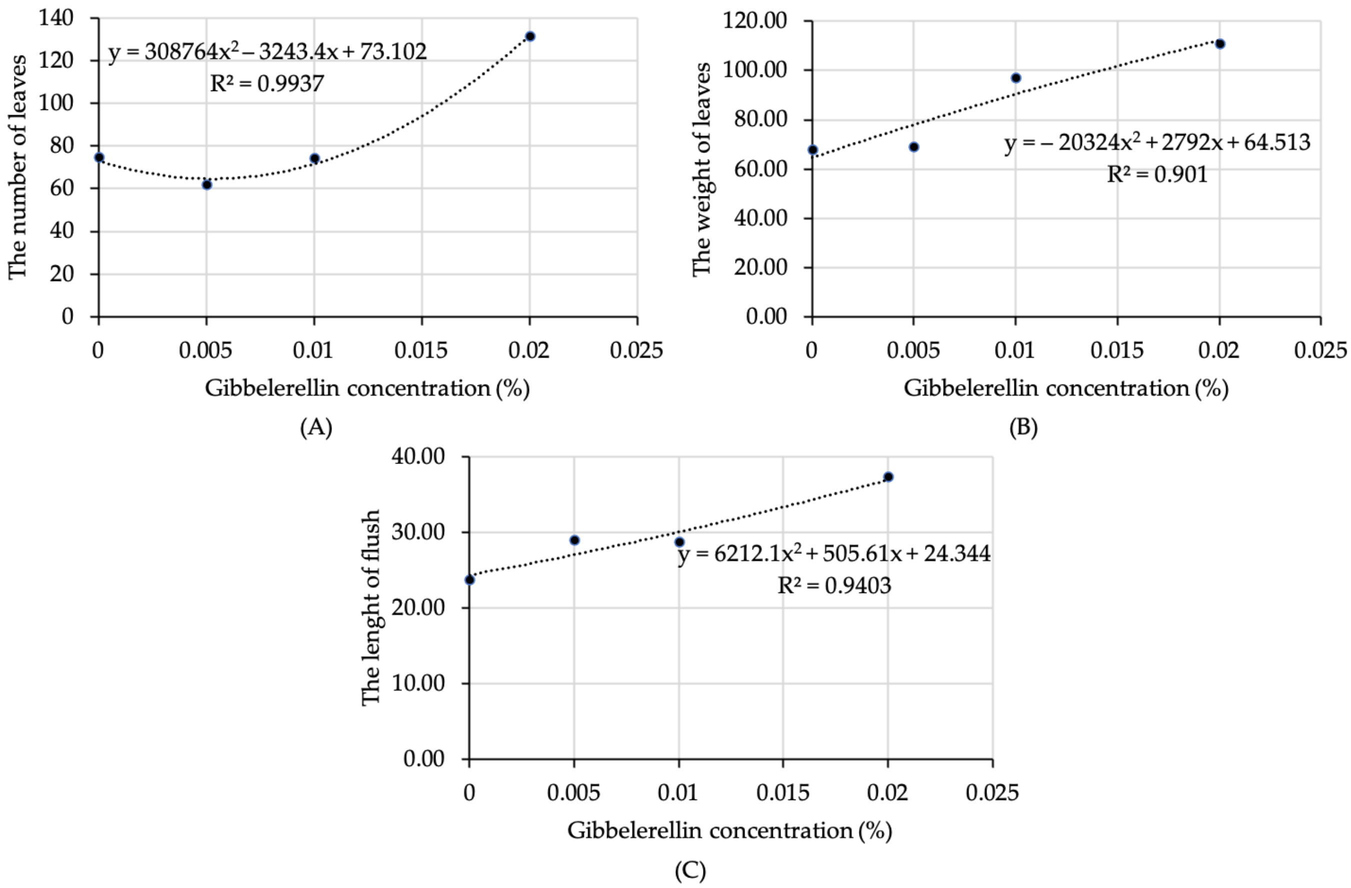

Figure 5. The mathematical equation derived from the regression model: gibberellin concentration and the number of leaves (**A**); gibberellin concentration; the weight of leaves (**B**); and the length of flush (**C**) in the unpruned leaves.

4. Discussion

During the first flushing period of June–July, there was a variation in growth due to pruning activity that was likely associated with differences in source capacity. In plants, the main sources are productive leaves, generally located outside of the canopy to obtain full sun and to photosynthesize normally, which produce photoassimilates for all of the plant organs; however, other green organs, such as green stems, green flowers, and green fruits, have also demonstrated potential as a photosynthetic site [46–49]. In major fruit-oriented citrus, such as mandarin, the roles of source and sink are closely attached to the leaf and fruit (as well as seed) organs, respectively [44]. In pruned kaffir lime plants, the initial source is carbohydrate reserves in the stems and roots, while the newly emerged flush becomes the sink; then, the formation of mature leaves on that flush becomes the source [43,45]. Source and sink interact to determine plant growth, yield development, and nutritional quality [50]. The communication of source and sink is regulated by plant hormones and nutrient status [51]. The good management of a source–sink balance could speed up the plant growth rate [52], including flushing performances.

Regarding flushing performance, pruning has a more dominant effect than gibberellin. Pruned plants immediately experience a reduction in source capacity, leaving only a 30 cm long main stem, whereas previously, it had a source in the form of a main stem as high as 75 cm and 75 leaves. The limited source capacity as the consequence of leaf removal in pruned plants caused a significant drop in growth performance [44,53], as indicated in the present experiment by a shorter flush, fewer flush numbers, and a smaller leaf size than the unpruned plants.

With or without gibberellin application, pruned plants will recover their lost foliage. Although it seems that pruning and gibberellins have a contrasting effect on plant height, the pruned plants could be said to grow faster because they increased the plant height by 21 cm (from 30 to 51 cm) in the sixth week, while the unpruned plants only increased by 10 cm (from 75 to 85 cm). The pruned plants restored 68% of the height and 49% of the leaf number, compared to the initial unpruned condition. The recovery process could not be completed up to 100% due to observations that were only made during the first flushing period. This is reasonable, as even previous research [54] has stated that mature orange trees need 9–11 months to recover the lost foliage and fine roots. In subsequent flushing periods, a greater percentage of recovery should be achieved, so that the gap between pruned and non-pruned plants can be narrowed. This indicates that the temporal aspect must be considered in interpreting the present finding. Moreover, the gap between pruned and unpruned growth and yield could be minimized under certain practices, such as shading. Artificial mild shading (24% light reduction) can increase chlorophyll B content and the photosynthetic rate, leading to increased kaffir lime leaf production [43].

The increase in kaffir lime leaf production in the present experiment is limited to the condition of grafting plant materials instead of seeds. In grafting, the type of rootstock has the potential to display differences in the growth response and yield of scion. The variability in rootstock in grafting is reportedly used in dwarfing citrus fruit trees [55] and increases tolerance to abiotic stresses, such as drought [56–58]. The present study only used rangpur lime (*Citrus limonia*) as the rootstock combined with kaffir lime as the scion. Moreover, earlier studies have confirmed the superiority of rangpur lime, which is tolerant to drought stress [59] and salinity [60]. There is no doubt that rangpur lime has become a popular species of citrus rootstock in Indonesia [7].

Grafted to rangpur lime, kaffir lime scion growth was boosted by the presence of 0.02% gibberellin and the absence of pruning. Multivariate analysis also confirmed a positive correlation between gibberellin and leaf production variables, similar to a previous study [61] which also revealed a positive correlation with the vegetative growth of mandarins. The supportive effect of gibberellin on vegetative growth is reasonable due to gibberellin's role in improving plant agronomic performance [62], including shoot proliferation [63], leaf expansion [64], and stem elongation [65,66], by inducing cell division and cell expansion [67]. The application of gibberellin resulted in broader and thinner leaves, irrespective of the various light level [68]. Exogenous gibberellin treatment improved the chlorophyll content and net photosynthetic rate, and also promoted the translocation of photosynthate from mature leaves (sources) into newly emerged buds (sink) [69]. Aside from supporting vegetative growth, the importance of gibberellin also includes its ability to regulate fruit growth [70], seed germination [71], and stress tolerance [72–75]. However, using a gibberellin concentration up to 0.02% has not been proven to increase the recovery speed of kaffir limes after leaf removal. The regression pattern showed the potential to increase the gibberellin concentration in future research.

5. Conclusions

There is a contrasting effect of exogenous gibberellins and pruning application on the leaf production of kaffir limes. Pruning showed a more dominant effect on the flushing response of kaffir lime plants. Pruning significantly reduced the flush number, flush length, and leaf size. Regarding its practical implications, applying 0.02% gibberellin significantly produced the longest flush, showing more and denser leaves, specifically in the absence of pruning. Correlation and regression analysis also confirmed similar findings. With the presence of pruning, the positive effect of gibberellin on leaf production is degraded due to the loss in foliage leading to a limited source capacity. The increase in gibberellin concentrations (>0.02%) needs to be explored in the future to determine whether it can speed up the foliage recovery of post-pruned kaffir limes.

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