Research article

Fruit and Seed Development of *Citrus aurantifolia* (Christm.) Swingle 'Pan Rampai' Cultivar

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seed maturation

Abstract

The 'Pan Rampai' cultivar of Mexican lime is highly consumed for its lime aroma and high juice content. However, occasional instances of low juice content could be found, potentially due to premature harvesting. Moreover, the seed development of the 'Pan Rampai' lime has never been investigated. Hence, this study was aimed to explore the development process of the 'Pan Rampai' lime fruit, focusing on its fruit weight, juice weight, juice content, fruit diameter, fruit length, fruit rind thickness, total soluble solids, titratable acid, TSS/TA ratio, and vitamin C content over the period of 11 to 18 weeks after flowering (WAF). Additionally, we examined the seed development by observing seed width, length, thickness, and germination percentage. Our findings revealed that fruit weight, juice weight, juice content, fruit diameter, and fruit length exhibited a consistent increase throughout the fruit development stages, while the fruit rind thickness decreased. Seed width and thickness remained relatively stable over 11 to 18 WAF, whereas seed length gradually increased. The study of germination percentage indicated that seed germination was mature from the 15th WAF, coinciding with the appearance of the secondary wall of seed coat. There results provide crucial insights into the optimal harvesting periods for juice (from 17 WAF) and seed (from 15 WAF) for the 'Pan Rampai' lime cultivar.

1. Introduction

Citrus aurantifolia (Christm.) Swingle, commonly known as the Mexican lime, is one of the most consumed acidic citrus fruits in the world. It belongs to the Rutaceae family. It is primarily cultivated in Mexico and others countries in South America, with some produce exported to the United States of America [1]. Additionally, Mexican lime cultivation is widespread in the West Indies and Egypt

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[2]. In Thailand, the Mexican lime holds the high cultivation and consumption rates, surpassing other varieties such as Tahitian lime and lemon. The lime plays a crucial role as a seasoning in Thai cuisine and beverages. The area dedicated to Mexican lime cultivation has consistently grown, expanding to approximately 17 thousand hectares, with an annual yield of about 165 thousand tons in 2022 [3]. Across Thailand, several cultivars of *C. aurantifolia* (Christm.) Swingle are grown, with 'Pan Rampai' being particularly popular among farmers and consumers. This cultivar is favored for its distinctive lime aroma and thin fruit rind, which facilitates juice extraction, making it a desirable variety with potential appeal to consumers worldwide. However, consumers often encounter the issue of low juice content when purchasing the lime. The variation in juice content can be a genetic factor or premature harvesting of the fruit. Each genetic variant possesses a unique profile that included juice content, which can be used to indicate maturity and thus proper time to harvest [4]. Citrus fruits typically undergo three stages of development. The initial stage (phase I) involves cell division, followed by the cell enlargement phase (phase II) in which water accumulation takes place. In the final stage (phase III), growth ceases, and the fruit begins to ripen [5]. Optimal harvesting for superior lime quality should occur towards the end of phase II when water accumulation is complete or at the onset of phase III. Late harvesting, during the latter part of phase III, lead to overly rapid fruit ripening and subsequent decline in the quality of lime juice due to decreased levels of pleasant volatile compounds [6]. Therefore, the study of the fruit development of Mexican lime 'Pan Rampai' can aid in determining the ideal harvesting stage and obtaining the highest lime quality.

In addition to physical changes during fruit development, it is crucial to observe the chemical properties such as total soluble solids (TSS), titratable acid (TA), and vitamin C content, as these parameters directly impact the quality of lime juice and its accumulation process throughout fruit development. Studies on analyzing TSS, TA, and vitamin C content in two orange species demonstrated an increase in TSS with a reduction in TA as the fruit matured [7]. Yang *et al*. [7] observed a slight increase in vitamin C content in two orange species, whereas Nagy [8] noted a decline in vitamin C content during maturation in three citrus varieties. However, the dynamics of TSS, TA, and vitamin C content during 'Pan Rampai' fruit development remains unexplored.

Furthermore, to improve commercial lime cultivars by achieving superior fruit quality with fewer seeds, thinner rinds, disease resistance and tolerance to abiotic stress, it is essential to breed and cultivate the lime. In this context, understanding lime seed development is of great significance when determining the optimal fruit harvesting time for seed collection before fruit abscission. Studies on seed development during fruit development in three citrus rootstocks revealed a positive correlation between seed size/weight and fruit size/weight [9]. Therefore, we aimed to elucidate the fruit and seed development in this study.

Overall, this study could contribute valuable insights into the optimal harvesting stage for 'Pan Rampai' lime in order to obtain the highest-quality fruit and address the issues of low juice content faced by consumers. Additionally, the chemical properties related to lime juice quality, including TSS, TA, and vitamin C content, during fruit development were also explored. Moreover, the study was aimed at providing a comprehensive understanding of seed development and germination in the 'Pan Rampai' lime, which holds significance for future breeding efforts and seed production of this cultivar.

2. Materials and Methods

2.1 Materials

Fruits of *C. aurantifolia* (Christm.) Swingle 'Pan Rampai' cultivar were obtained from the Tropical Fruit Research and Development Center (TFRDC), Department of Horticulture, Faculty of

Agriculture at Kamphaeng Sean, Kasetsart University. Eight 'Pan Rampai' trees were cultivated in 80 cm-diameter cement rings with a 2.5 m spacing between trees and a 3 m spacing between rows. Fertilizer was applied at 100 g per plant every month on soil using an NPK fertilizer 21-7-14, and spray fertilizer was applied on the plants every two weeks with NPK fertilizer 18-6-12, along with micronutrients and trace elements. Fruit samples ranging from one to twenty-two fruits each week after flowering (WAF) were collected from 11 to 18 WAF counting from flowers that bloomed from the first week of August 2018 until the last week of September 2018 (8 weeks). All the fruits from each week were harvested on December 11, 2018, when the fruits from the first flowering reached 18 WAF.

2.2 Fruit quality evaluation

The following parameters were evaluated to assess the quality of the fruits from 11-18 WAF:

(1) Fruit and juice weight were measured using a scale with two-decimal places (Adventurer^{TM} OHAUS, New Jersey, USA).

(2) The juice content was calculated as the percentage of juice weight divided by fruit weight.

(3) The fruit diameter, fruit length and fruit rind thickness were measured in triplicate for each fruit using a digital vernier caliper in millimeters (INSIZE, Suzhou New District, China).

(4) Total soluble solids (TSS) of 'Pan Rampai' lime juice after filtration with filter cloth were measured using a digital refractometer (PAL-1, ATAGO, Tokyo, Japan).

(5) Titratable acidity (TA) was analyzed following the protocol from the Association of Official Analytical Chemists [10]. This involved using 0.1N sodium hydroxide as the titrant, 2 mL of lime juice as the analyte, and 1% phenolphthalein as the indicator. The TSS/TA ratio was then calculated.

(6) Vitamin C content was analyzed using the titration method [10]. It involved adding 2 mL of lime juice to an extracting solution (3% oxalic acid, 8% acetic acid) as the analyte and using 2,6-dichlorophenol indophenol as the titrant. The standard was a 1 mg/mL standard ascorbic acid solution.

2.3 Seed extraction and seed quality evaluation

Seeds were extracted from the lime fruits. Seed width, seed length and seed thickness were measured using a digital vernier caliper in millimeters (INSIZE, Suzhou New District, China). The seeds were extracted from fruits of each week. The number of seed for germination test was counted. Then, germination test of seeds was done in peat moss. Seed germination was observed at 8 weeks after sowing. The number of normal seedlings were counted, and the germination percentage was calculated.

3. Results and Discussion

3.1 The fruit growth of 'Pan Rampai' lime

The growth parameters of 'Pan Rampai' lime fruits, including fruit diameter, fruit length and fruit weight, showed a gradual increase over the 11-18 WAF period.

Fruit diameter and fruit length: The fruit diameter reached its peak at 18 WAF, while the fruit length remained stable at 17 WAF (Figure 1a, b). These findings suggest that the growth of 'Pan Rampai' lime from 11 to 17 weeks after flowering corresponded to phase II of citrus fruit

development which is the water accumulation and cell enlargement stage. From 17 to 18 weeks after flowering, the fruits entered phase III, during which they ceased growing and the ripening process was initiated [5]. The increase in fruit size followed a sigmoidal curve pattern similar to the fruit growth curves observed in other citrus fruits such as the sweet orange, Kinnow, and Feutrall's early [11].

Fruit weight, juice weight, and juice content: The fruit weight, juice weight, and juice content of 'Pan Rampai' lime also showed a gradual increase throughout the fruit developmental stage, entering a stationary phase at 17 WAF (Figure 1c, d). The juice content of the 'Pan Rampai' cultivar reached the steady phase at 17 WAF, which aligned with a previous study on mandarins where the juice content increased after the fruits reached maturity [12].

Fruit rind thickness: As the fruit size increased, the fruit rind thickness decreased (Figure 1e). This finding was consistent with a study on citrus by Lu *et al*. [13] that reported a decrease in fruit rind thickness as fruit size increased. Notably, while the fruit weight and juice weight increased in parallel, the juice content calculated as the percentage of juice weight divided by fruit weight continued to increase. This may have been attributed to changes in the structure of the juice sac, allowing the accumulated juice to be more easily squeezed out. Furthermore, the thinner fruit rind at the mature stage may have contributed to higher juice content. Based on this study, the optimal harvest time for 'Pan Rampai' lime fruits appears to be from 17 or 18 WAF.

3.2 The chemical properties of 'Pan Rampai' lime juice

In this study, we analyzed the vitamin C content, titratable acid (TA), total soluble solids (TSS) and the TSS/TA ratio of 'Pan Rampai' lime juice during the fruit developmental stage from 11 to 18 WAF. The results demonstrated a steady accumulation of all these components (Figure 2). The TSS/TA ratio indicated that 'Pan Rampai' lime fruit was acidic, which was consistent with previous findings in another cultivar of Mexican lime [14]. Studies on lemon and orange have shown a decrease in vitamin C content as fruit size increases during fruit development [15]. In contrast, the vitamin C content in 'Pan Rampai' lime remained steady throughout development, possibly due to its stable TA content (Figure 2a, b). This finding was supported by the fact that citric acid, the main acid in lime, can stabilize vitamin C [16].

The steady accumulation of TSS and TA observed in this study was consistent with the findings of Karishma *et al*. [17] on mandarins at different stages of fruit development, where TSS, TA, and vitamin C content did not show significant variations as the lime fruits accumulated these components throughout the developmental stage. Similarly, Albertini *et al.* [18] studied acidic and acidless citrus and found that in acidic citrus, acid accumulation remained steady during the second half of phase II, which corresponded to the phase of the samples in this study. In several studies on oranges or acidless citrus, an increase in TSS and a decrease in TA were observed in the developmental stage at the near ripening stage [7, 18]. The accumulation of acid in lime is facilitated by the mechanism of V-ATPase in acid citrus, which enables the tolerance of different pH levels and facilitates the pumping of H^+ from the cytosol to the vacuole [19]. Furthermore, there is supporting evidence that a P-type proton pump gene, an integral membrane protein responsible for pumping proton (H^+) across membranes and regulating acid accumulation in vacuoles, is highly expressed in correlation with citrus fruits that are high in citric acid [20]. These findings suggest that if limes are harvested at mature stage (17 to 18 WAF), the chemical properties of the lime will not differ. However, it is important to consider that there are other factors influencing fruit quality, such as season, and preharvest and postharvest management [14, 21].

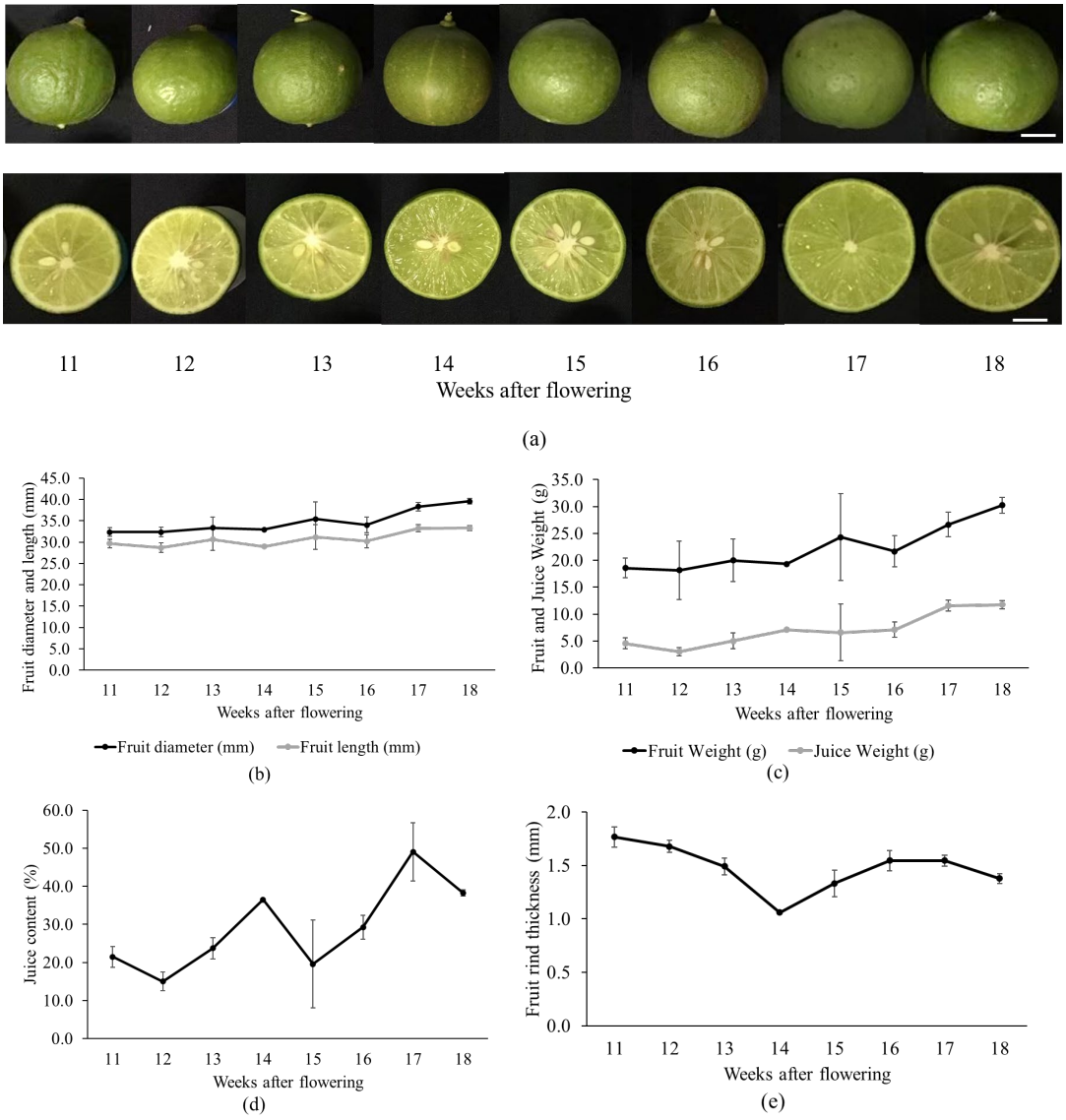

Figure 1. Fruit development of 'Pan Rampai' lime cultivar. (a) External and internal structure of 'Pan Rampai' fruit from 11 to 18 weeks after flowering. Scale bar = 1 cm (b) Fruit diameter and fruit length (mm). (c) Fruit weight and juice weight (g). (d) Juice content (%). (e) Fruit rind thickness (mm) of 'Pan Rampai' lime cultivar from 11 to 18 weeks after flowering. The error bar represents the standard error.

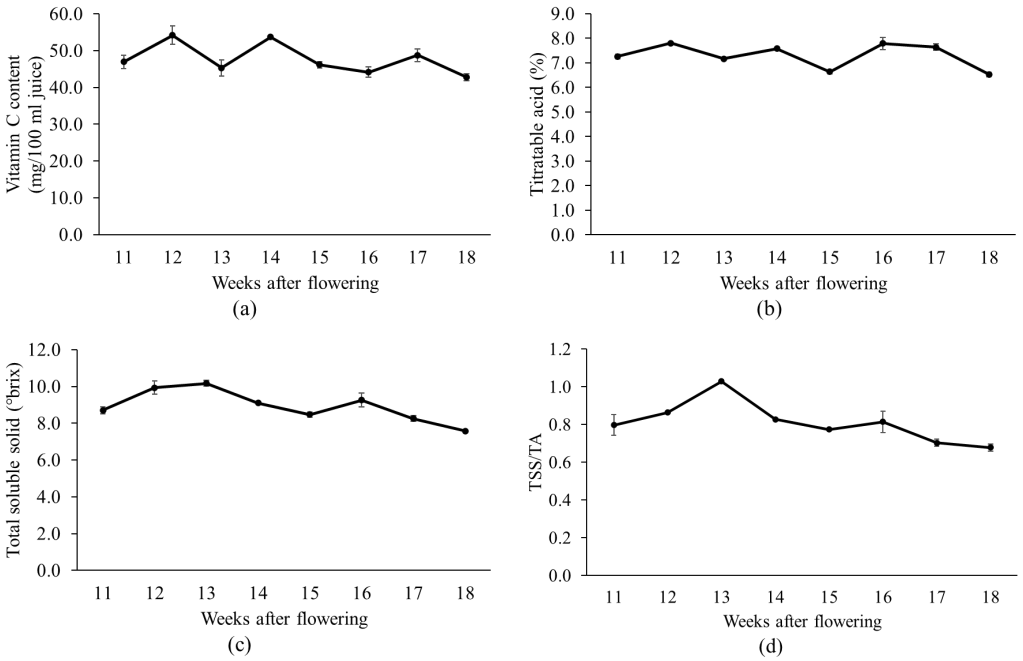

Figure 2. The chemical properties of 'Pan Rampai' lime juice during the development of lime fruit from 11 to 18 weeks after flowering. (a) Accumulation of vitamin C content in lime juice during 11 to 18 weeks after flowering (mg vitamin C/100 ml juice). (b) Accumulation of titratable acid (TA) in lime juice (%). (c) Accumulation of total soluble solid (TSS) in lime juice (°brix). (d) TSS/TA ratio of 'Pan Rampai' lime cultivar from 11 to 18 weeks after flowering. The error bar represents the standard error.

3.3 The correlation of fruit qualities

Strong positive correlations were determined between fruit length and fruit diameter, fruit weight and fruit diameter, juice weight and fruit diameter, fruit weight and fruit length, juice weight and fruit length, juice weight and fruit weight, juice content and fruit weight, and juice content and juice weight. There were moderate correlations between juice content and fruit diameter, and juice content and fruit length, as they gradually increased along the fruit growth (Table 1, Figure 1b, c, and d).

There was a strong correlation between TSS/TA and TSS, as the TSS/TA value had been calculated from TSS (Table 1). There was a moderate correlation of TA and vitamin C, where vitamin C is ascorbic acid, a type of acid accumulated in lime juice (Table 1). The moderate correlation between TSS and TA was probably because TA is a type of soluble solid that can be detected in TSS (Table 1).

TSS showed a strong negative correlation with fruit diameter and juice weight, and there was also moderate negative correlation between TSS/TA and fruit diameter, vitamin C and fruit length, TA and fruit length, TSS and fruit length, TSS/TA and fruit length, TSS and fruit weight, TSS/TA and fruit weight, TSS/TA and juice weight, TSS and juice content, and TSS/TA and juice content. The increase in fruit size or weight with TSS decrease was also observed in apples [22]. This might be explained by the increase in size and juice content of the fruit leading to an accumulation of water inside the fruit. This accumulation of water may have caused dilution of the TSS, TA and vitamin C, as observed in Figure 1b, c and d and Figure 2.

Table 1. Correlations among the fruit quality parameters of 'Pan Rampai' lime.

	Fruit Diameter	Fruit Length	Fruit Weight	Juice Weight	Juice Content	Fruit Rind Thickness	Vitamin C	TA	TSS	TSS/ TA
Fruit diameter	1.00									
Fruit length	0.96	1.00								
Fruit Weight	0.87	0.78	1.00							
Juice Weight	0.93	0.88	0.93	1.00						
Juice content	0.70	0.67	0.85	0.89	1.00					
Fruit rind thickness	-0.19	-0.07	-0.22	-0.34	-0.36	1.00				
Vitamin C	-0.49	-0.60	-0.36	-0.40	-0.11	-0.14	1.00			
TA	-0.49	-0.51	-0.16	-0.31	0.05	0.26	0.60	1.00		
TSS	-0.80	-0.73	-0.67	-0.80	-0.57	0.20	0.40	0.56	1.00	
TSS/TA	-0.71	-0.58	-0.66	-0.72	-0.54	0.08	0.16	0.25	0.92	1.00

3.4 Seed development of 'Pan Rampai' lime

The size of 'Pan Rampai' lime seeds showed an increase in seed length each week, while the seed width and thickness remained relatively stable (Figure 3a). This change in the dimension of 'Pan Rampai' lime seeds was similar to the observations of Koltunow *et al*. [23] in Valencia, where seed size increased along with fruit size.

The seed germination percentage was found to be very low at 11 and 13 WAF, with no germination observed at 12 and 14 WAF. However, starting from week 15 after flowering, there was a noticeable increase in seed germination (Figure 3b). Upon observing the seeds at 11-12 WAF, it was noted that they were still very young, and the seed coat had not fully developed. Seeds from week 13-14 after flowering began to develop a secondary wall in the seed coat, which resulted in a low germination percentage. By week 15 after flowering, the seeds exhibited a light brown color, indicating the presence of a fully developed secondary wall and signifying seed maturity (Figure 3c). The development of a secondary wall in the seed coat is a characteristic feature of mature seed [24]. If the 'Pan Rampai' lime is to be used in a breeding program or employed as a rootstock for further study, it is recommended that the fruit be harvested and the seeds be extracted at a minimum of 15 WAF to achieve a higher germination rate. However, it is worth noting that some seeds germinated at week 11 and 13. Based on the observations, some of these seeds had light brown seed coats.

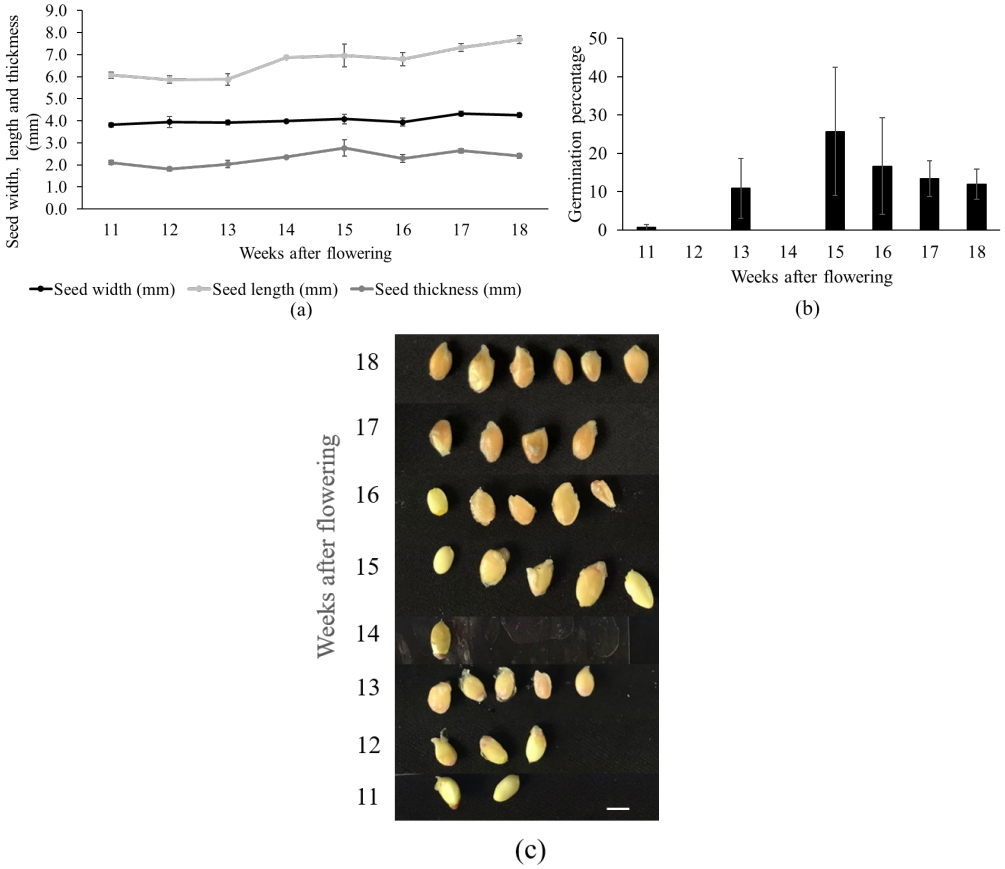

Figure 3. Seed development of 'Pan Rampai' Lime. (a) Seed width, seed length and seed thickness (mm) (b) Germination percentage (c) Appearance of seeds (Scale bar = 0.5 cm). of 'Pan Rampai' lime cultivar's seeds from 11 to 18 weeks after flowering. The error bar represents the standard error.

4. Conclusions

In this study, the physical and chemical qualities of 'Pan Rampai' lime during fruit development were analyzed. The results revealed an increase in size and weight of the fruit, accompanied by a decrease in fruit rind thickness. The juice content reached its maximum at 17 WAF, indicating that the fruit had reached maturity and could be harvested from this stage onwards. The levels of vitamin C, TSS, and TA and the TSS/TA ratios remained relatively stable throughout the developmental stages examined in this study. The seed development of 'Pan Rampai' lime showed signs of entering the maturity stage at 15 WAF. Therefore, for breeding programs or for use as a rootstock, it is recommended to harvest the fruit and extract the seeds no earlier than 15 WAF. This finding can be implemented for proper Mexican lime harvesting for juice consumption or for seed extraction for root stock preparation or breeding programs. Future studies on other citrus fruits or variables could benefit from a similar approach to elucidate their developmental patterns.

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